THE UNIVERSITY *of* EDINBURGH

Geochemical Associations and Availability of Cadmium (Cd) in a Paddy Field System, Northwestern Thailand

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PhD

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2014

Declaration

I certify that the work described in this thesis in my own, except where otherwise stated, and has not been previously submitted for any other degree at this, or any other university.

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Abstract

The Mae Tao watershed, northwestern Thailand, has become contaminated with cadmium (Cd) from the zinc mining activities area in the nearby Thanon-Thongchai mountains. Consumption of Cd-contaminated rice has led to documented human health impacts. The aim of this study was to clarify transfer pathways from creek and canal waters to the paddy field soils near Baan Mae Tao Mai village and to determine the relationship between Cd forms in the soil and uptake by rice plants. Soils, irrigation canal sediments and water samples were collected during the dry season and at the onset of the rainy season. Rice samples were collected at harvesting time and samples of soil fertiliser were also obtained. Water samples were filtered, ultrafiltered and analysed by ICP-MS whilst sub-samples of dried, ground soils and sediments were first subjected to micro-wave assisted acid digestion (modified US EPA method 3052). XPRD and SEM-EDX methods were used for mineralogical characterisation and selective chemical extractions have assisted in the characterisation of solid phase Cd associations.

Transfer mainly occurred in association with particulate matter during flooding and channel dredging and, in contrast with many other studies, most of the soil Cd was associated with exchangeable and carbonate-bound fractions. Moreover, there was a significant positive relationship between soil total Cd and rice grain Cd (R^2=0.715), but a stronger relationship between both the Tessier-exchangeable soil Cd and the BCR-exchangeable soil Cd and rice grain Cd (R^2=0.895 and 0.861, respectively). Stable Isotope Exchange (gives isotopically exchangeable Cd - E value) is generally considered to provide a better measure of bioavailability. The results of this study showed that SIE gave values which significantly positive correlated with the Tessier exchangeable fraction.

Biochar has a porous structure and acidic functional groups on its surface which give it a high capacity to affect heavy metal adsorption when added to soils. Biochar

samples were produced from rice husk (RHC) and miscanthus (MC) since these are cheap and readily available materials in Thailand. Indeed rice husk char is already used for rice seed germination by local farmers. Evaluation of the chemical and physical properties of the chars showed that the lower temperature (350°C) chars had greater proportions of oxygen-containing functional groups than those produced at 700°C. Moreover the low temperature RHC had the greater cation exchange capacity than the MC produced at the same temperature. In abiotic feasibility tests, it was shown that RHC was more effective than MC at lowering soil available Cd. The former reduced the E values for the medium-Cd soils by 4.7% and 16.0% when 1% and 5% RHC, was added, respectively. From pot experiments, in medium-Cd soils, 1% and 5% RHC amendment showed the potential to decrease Cd uptake by rice roots. However, further work involving addition of a greater proportion of biochar with a higher number of replicates is needed to reach more robust conclusions.

Lay Summary

The Mae Tao watershed, northwestern Thailand, has become contaminated with cadmium (Cd) as a result of zinc ore extraction (Padaeng deposit) in the nearby Thanon-Thongchai mountains. Consumption of contaminated rice has led to documented human health impacts. The aim of this study was to elucidate transfer pathways form creek and canal waters to the paddy field soils near Baan Mae Tao Mai village and to determine the relationship between Cd speciation in the soil and uptake by rice plants. Transfer mainly occurred in association with particulate matter during flooding and channel dredging and, in contrast with many other studies, most of the soil Cd was associated with exchangeable and carbonate-bound fractions. Moreover, there was a linear relationship between soil total Cd and rice grain Cd (R^2=0.715), but a stronger relationship between both the Tessier-exchangeable soil Cd and the BCR-exchangeable soil Cd and rice grain Cd (R^2=0.895 and 0.861, respectively). Stable Isotope Exchange (gives exchangeable-E value) is generally considered to provide a better measure of bioavailability;, the results of this study showed that SIE gave values which matched well with the Tessier exchangeable fraction. The rice husk chars (RHC), great capacity in cation absorption and available, were used and showed the promising properties with porous structure and high CEC. In feasibility test, RHC can reduce the E values from the medium-Cd soils 5.7% and 20.2% from 1% and 5% RHC added. From pot experiment, in medium-Cd soils, 1% and 5% RHC amendment showed the potential in decreasing the available Cd in soils and in also roots significantly.

Acknowledgements

Initially, I would like to thank the Royal Thai Government Scholarship that gave me the opportunity to undertake my PhD study in Scotland.

Foremost, I offer my sincerest gratitude to Dr Margaret Graham, my supervisor, Prof John Farmer, my co-supervisor and also many thanks to Dr Lorna Eades, Dr Joanna Cloy, Dr Helfrid Rossiter, Dr Dusan Uhrin and Dr Ian Oliver for their academic advice and support throughout this project. I also express my thanks to Dr Kate Heal and Dr Christine Davidson, my PhD examiners, for their kindness and recommendations.

I also offer my appreciation for technical support from Dr Nicholas Odling (XRPD) and Dr Nicola Cayser (SEM-EDX). Ialso wish to thank Dr Ondrej Masek for production of rice husk and miscanthus biochars and for providing helpful knowledge about the properties of biochar.

Moreover, this project would have been accomplished without the support of Innovative Initiative Grant from the University of Edinburgh Development Trust.

During the samples collection in Thailand, I do thank you the Mae Sot civil society and especially Mr Boonruang Tawong, the paddy fields owner, for their kindness, advice and much help during fieldwork. I also thank Dr Avorn Opatpatanakit, Dr Choochad Santasup and their team at the Faculty of Agriculture, Chiangmai University for their academic support during fieldwork and samples collection.

I would like to thank my colleagues throughout four years, David Blair, Cecilia Xu, Kenneth Macgregor, Ellen Hue, Nicholle Bell, Tara Dufty and Robert Grey from their warm welcome and lovely farewell party. So do other friends who worked hard together in room 293, Joseph Black Building.

Finally, I would like to thank my family and friends both in UK and Thailand for their best wishes, support and love.

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Chapter 1 Introduction

1.1 Overview

Cadmium (Cd) has a crustal abundance of only 0.15 mg kg^{-1} and as such is a relatively rare element in nature. It is extremely toxic and has no known function in the human body (WHO, 1996; Fraga, 2005). Exposure of workers in the early-mid 20th Century was common in many industrial settings where (i) processes such as electroplating or spray painting; (ii) production of batteries or fertilisers took place. Environmental contamination also resulted from (i) the release of industrial effluents, e.g. Ni-Cd battery plants; (ii) application of sewage sludge; (iii) use of phosphate fertilisers; (iv) non-ferrous metal mining, e.g. zinc mining (Jung and Thornton, 1996; Sloan *et al.,* 1997; McLaughlin *et al*., 1999; Durza, 1999). As a consequence of such anthropogenic processes, Cd concentrations increased, especially in soils and waters in the vicinity of industrial and agricultural areas. This has led to contamination of foodstuffs which in turn has, in some cases, led to severe impacts on human health. A well-documented contamination incident occurred in Japan in the early part of the 20th Century. The Kamioka Mine (Mitsui Mining and Smelting Company) in Toyama Prefecture, central Japan, released Cd-containing wastes into the Jinzu River basin. Initially, fish in the river started to die, and the rice irrigated with contaminated water did not grow well. The rice absorbed the cadmium from the water and then the Cd accumulated in the people eating the contaminated rice (e.g. Kaji, 2012). The Cd poisoning caused softening of the bones which caused severe pain in the bones and joints and this led to the first documented cases of Itai-Itai ("ouch ouch") disease. Subsequently, additional health issues including kidney failure were also attributed to Cd exposure.

In many developed countries, permissible human exposure levels to Cd have been markedly reduced and industrial emissions are now strictly controlled. However, in

developing countries, large-scale resource exploitation started much later and practices associated with resource recovery have often not been so tightly regulated. As a consequence, agricultural areas in developing countries have been increasingly impacted by contaminants such as Cd and effects on human health have only been noticed very recently. For example, in Thailand, several decades after the advent of zinc mining, high accumulation of Cd in rice growing areas was reported only in the early 2000s. Subsequent investigations in the mid-2000s revealed that a high proportion of local people who regularly consumed rice from the contaminated paddy fields had serious health problems (Simmons *et al.*, 2005; Swaddiwudhipong *et al.*, 2011).

In order to understand how to limit Cd uptake by rice plants it is necessary to determine its geochemical associations within the paddy soils. To do this, it is useful to understand the nature of the source material in the mining area and how it has been transformed during transport in irrigation waters and eventually onto the paddy soils.

This study aimed to: (i) characterise Cd geochemical transformation from the source through the waterways to the paddy soils; (ii) investigate the factors affecting those changes, especially those controlling Cd speciation; and importantly (iii) evaluate practical amendments to reduce the level of Cd in rice grains with a view to recommending remediation strategies and limit human exposure to Cd.

1.2 Cadmium (Cd)

This section gives a brief outline of key physical and chemical properties of Cd, its uses, its natural occurrence and sources of anthropogenic contamination.

1.2.1 Properties of Cd

Cd is a soft, silver-white, ductile and malleable metal that is grouped within the transition elements together with zinc and mercury (Greenwood and Earnshaw, 1997). Seven isotopes of Cd and one radioactive isotope have been reported to occur naturally (Table 1.1). The stable isotopes and their relative amounts are: Cd-106 (1.3%), Cd-108 (0.9%), Cd-110 (12%), Cd-111 (13%), Cd-112 (24%), Cd-114 (29%), and Cd-116 (7.5%). The only radioactive isotope is Cd-113 (12%) and it has an extremely long half-life of ~9.3×10^{15} y (Aroma National Laboratory, 2005).

With respect to its physical properties, Cd has relatively low melting and boiling points (Table 1.1). When heated, elemental Cd is rapidly oxidized to Cd oxide which then combines with reactive gases such as carbon dioxide, water vapour, sulphur dioxide, sulphur trioxide or hydrogen chloride to form Cd carbonate, hydroxide, sulphate or chloride, respectively. Many inorganic Cd compounds, e.g. chlorides ($CdCl_2$) and sulphates ($CdSO_4$), are highly soluble in water but another group including Cd oxide (CdO), carbonate ($CdCO_3$) and sulphide (CdS) are almost completely insoluble (Greenwood and Earnshaw, 1997). As suggested by the ionisation energies shown in Table 1.1, Cd metal readily loses two electrons and thus exists mainly in the +II oxidation state, e.g. $Cd^{II}O$, $Cd^{II}CO_3$, $Cd^{II}S$. Compounds where Cd is in the +I state have been produced, e.g. $Cd_2(AlCl_4)$ (formed by dissolving Cd in a mixture of Cd chloride and Al chloride).

Table 1.1 Selected Properties of Cd

Property		**Cd**
Atomic number		48
Number of naturally occurring isotopes		8
Atomic weight		112.411(8)
Electronic configuration		$[Kr]4d^{10}5s^2$
Electronegativity		1.7
Metal radius (12 coordinate)/pm		151
Effective ionic radius/pm	II	95
	I	-
Ionization energies/kJ mol^{-1}	1^{st}	876.5
	2^{nd}	1631
	3^{rd}	3644
E° (M^{2+}/M)/V		-0.4030
MP/ ˚C		320.8
BP/ ˚C		765
Density (25˚C) /g cm^{-3}		8.65
Electrical resistivity (20˚C)/ μohm cm		7.5

The half-life of $9.3\pm1.9 \times 10^{15}$ y for ^{113}Cd is the longest known for any β–emitter; note that this is 2 million times the age of the earth (4.6×10^9 y)

From: Greenwood and Earnshaw (1997)

1.2.2 Uses of Cd

Pigments such as Cd yellow (CdS), Cd orange (solid solution of CdS and Cd selenide (CdSe), and Cd red (solid solution of CdS and CdSe) were developed for use in artists' paints and for architectural decoration in the 1840s because there were very few strong colours in the yellow-orange-red part of the spectrum. Indeed, cadmium yellow was one of the pigments used by Post-Impressionist painters (Opila *et al.*, 2012). These Cd-containing pigments were particularly attractive because they stand up to high stresses and high temperatures. They retain their colour throughout the

lifetime of the glass, plastic, ceramic or enamel into which they have been incorporated (www.cadmium.org, 2009). Use of Cd pigments has, however, declined in recent years and accounts for <10% of total Cd use (Morrow, 2010).

A major (~86% globally) use of elemental Cd is now in rechargeable Ni:Cd batteries which contain a positive Ni oxide-hydroxide electrode and a negative Cd electrode which are separated by a solution of potassium hydroxide (Bernard, 2009). Due to its ability to resist corrosion, Cd is also used to protect steel components, e.g. of aircraft, and electroplating accounts for ~6% of global use (Morrow, 2010). Other uses have included blue and green phosphors in colour television tubes and alloys (80% Ag / 15% In / 5% Cd) to control neutrons in nuclear reactors (Scoullos *et al.*, 2001).

1.2.3 Natural Occurrence of Cd

Cadmium is widely distributed in the environment but is generally present at very low concentrations. It is listed as the 64th most abundant element in the periodic table and as such is considered to be a trace element. Table 1.2 summarizes its natural occurrence in various compartments of the environment. For example, Cd concentrations are typically 0.1 mg kg^{-1} in the Earth's crust, 0.001 µg L^{-1} in surface sea water, <1 µg L^{-1} in clean fresh water and 0.35 mg kg^{-1} in clean soils (Cox, 1995). Table 1.2 also shows typical concentrations of Cd in contaminated waters and soils. These are often up to 4 orders of magnitude higher than those in natural waters and 5 orders of magnitude greater than background concentrations in soils.

Table 1.2 Cd Concentrations in the Environment

Location	**Concentration**
Crust	0.1 mg kg^{-1}
Sea water:	
surface	0.001 µg L^{-1}
deep ocean	0.1 µg L^{-1}
polluted coasts	Up to 15 µg L^{-1}
Fresh water:	
clean	< 1 µg L^{-1}
polluted	1-10 mg L^{-1}
mining areas	100-700 µg L^{-1}
Drinking water (guideline value *)	3 µg L^{-1}
Soils:	
clean	0.35 mg kg^{-1}
polluted	Up to 1000 mg kg^{-1}
Edible plants	0.05-2 mg kg^{-1}
Human body:	
average	0.7 mg kg^{-1}
kidney	10-30 mg kg^{-1}
liver	2-3 mg kg^{-1}

From Cox (1995)

* Guidelines for Drinking-water Quality (WHO, 2008)

In terrigenous systems, the main naturally occurring mineral ore containing Cd is greenockite (CdS). It was first recognized in Scotland when a tunnel was cut for the Glasgow-Paisley-Greenock railway in 1840 and it was named after the land owner, Lord Greenock (Handbook of Mineralogy, 2005). Other minor minerals are octavite (CdSe) and monteponite (CdO) (Kabata-Pendias and Mukherjee, 2007).

Cd in the form of CdS is commonly found in the natural environment in association with Zn ores (e.g. sphalerite) (USGS, 2013). Thus Cd is usually found in the same places as Zn and it is Zn ores with a high Cd/Zn ratio that are commonly used in the commercial recovery of Cd. Indeed, over 95% of Cd is extracted from Cd-enriched by-products resulting from the roasting of Zn minerals. The largest producers of Cd are China, Japan, Korea, Mexico, the United States, the Netherlands, India, the United Kingdom, Peru, and Germany, where Zn ores are mined (USGS, 2002). However, in other Zn-enriched areas such as Thailand, where Cd is present as an impurity in smithsonite ($ZnCO_3$), Cd recovery is not economically viable due to the low Cd/Zn ratio of the Zn ores (Stoeppler *et al*., 1991 and Mineral Information Institute, 2009). It is also present as an impurity in phosphate minerals (Mineral Information Institute, 2009).

1.2.4 Natural and Anthropogenic Sources of Cd in Soils and Water

There are a number of natural and anthropogenic processes which add Cd to soils and waters. Natural sources include volcanic eruptions but the amount of Cd released is usually small in comparison to man-made sources. The latter include wastes from factories, cement manufacturing, waste incineration, fossil fuel combustion, sewage sludge, phosphate fertilizers and, of most importance, nonferrous mining, especially Pb and Zn mining, which is the biggest input source of Cd to soils (WorldBank, 1998) (Figure 1.1).

In Europe, phosphate fertilizers are the major anthropogenic sources of Cd (54-58%). Atmospheric deposition contributes about 39-41% and 2-5% comes from sewage sludge. The average Cd concentration in European topsoil is ~0.28 mg kg^{-1}, slightly higher than concentrations in the subsoil (Alloway, 2005; Pan *et al.*, 2010).

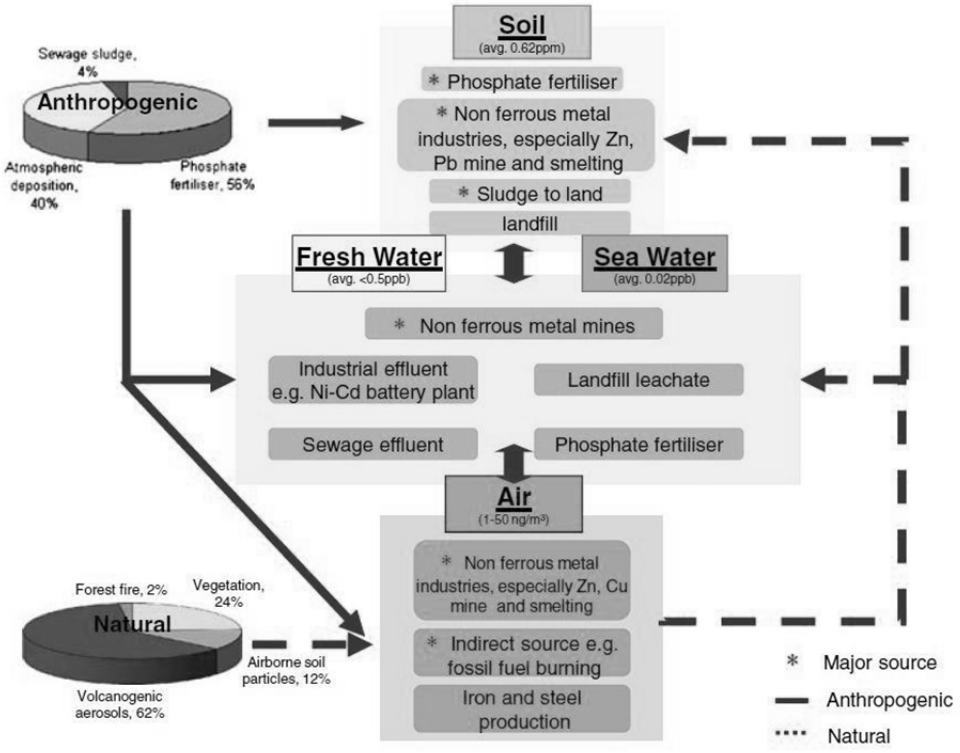


Figure 1.1 General source-pathway model for Cd (Pan *et al.*, 2010)

1.3 Environmental Behaviour of Cd

1.3.1 Speciation of Cd in Natural Waters

Cadmium is a soft Lewis acid and as such forms complexes with soft Lewis bases like Cl^- and OH^-. Common aqueous phase species are therefore $CdCl^-$, $CdOH^-$, $CdHCO_3^+$, $CdCl_3^-$, $CdCl_4^{2-}$, $Cd(OH)_3^-$ and $Cd(OH)_4^{2-}$ together with organic complexes (Alloway, 1995). More specifically the speciation of Cd in natural waters is affected by factors such as pH and the presence of complexing ligands which in turn is influenced by E_h.

Figure 1.2 shows the dependence of Cd speciation on pH in pure water. This shows that Cd^{2+} is the main species present in solution under acidic conditions. In the pH range 10-12, the hydrolysis products $Cd(OH)^{+}$ and $Cd(OH)_2^{0}$ become the most significant species in solution whilst the negatively charged $Cd(OH)_3^{-}$ and $Cd(OH)_4^{2-}$ dominate beyond pH 12 (Figure 1.2). When a mixture of ligands such as chloride, sulphate, and inorganic carbon is present, the free Cd^{2+} ion contributes ~80% of dissolved Cd^{II} below pH 8.5. The remainder is in the form of complexes with chloride and sulphate. Between pH 8.5 and pH 10.5, carbonate complexes dominate and at pH>10.5, hydrolysis products are the main form of Cd in solution (Figure 1.3) (Wilkin, 2007).

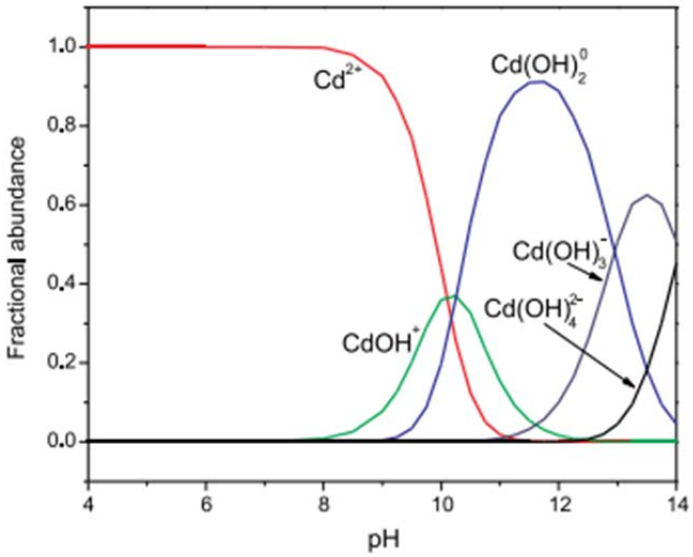


Figure 1.2 Species distribution of Cd(II) in pure water as a function of pH at 25 °C (Wilkin, 2007)

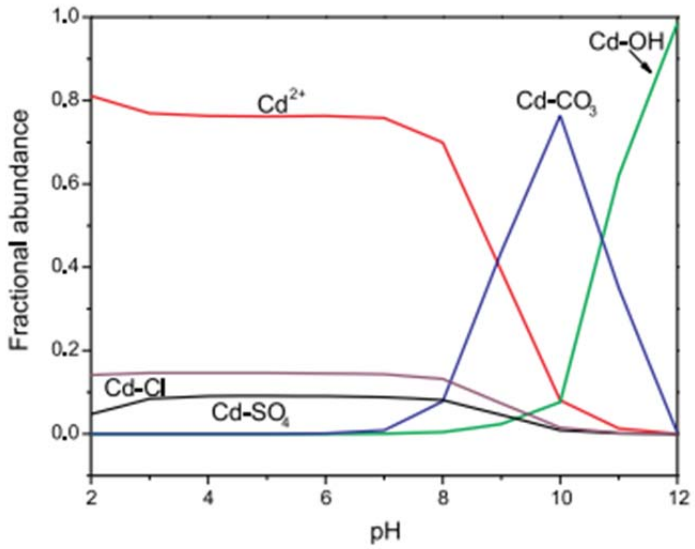


Figure 1.3 Cadmium speciation as a function of pH in solution containing chloride (100 mg L^{-1}), sulphate (100 mg L^{-1}), and inorganic carbon (100 mg L^{-1}). Cadmium chloride complexes include $CdCl^+$ and $CdCl_2^0$. Cadmium sulphate complexes include $CdSO_4^0$ and $Cd(SO4)_2^{2-}$. Cadmium complexes with inorganic carbon include $CdCO_3^0$, $CdHCO_3^+$, and $Cd(CO3)_2^{2-}$. Cadmium hydroxyl complexes include $CdOH^+$, $Cd(OH)_2^0$, and $Cd(OH)_3^-$. Total cadmium is equal to 1 mg L^{-1}. (Wilkin, 2007)

Under anoxic conditions, the nature of certain ligands will change. Of particular importance with respect to Cd is the conversion of sulphate to sulphide as E_h decreases. When sulphide activity exceeds $10^{-12.9}$ M, this is often accompanied by the transfer of Cd from the aqueous phase to the solid soil (Christensen and Huang, 1999). This will be discussed further in Section 1.3.2.

1.3.2 Processes Controlling the Distribution of Cd between the Aqueous Phase and Soil Solids

Adsorption/desorption, precipitation and co-precipitation appear to be the most important processes controlling the partitioning of Cd between inorganic solid and

soluble forms at the concentrations normally encountered even in the majority of polluted soils (Christensen and Huang, 1999; EPA, 1999).

Cadmium may associate weakly, e.g. by electrostatic forces, with solid surfaces and this is described as non-specific adsorption. Alternatively, it may be held at ion exchange sites or by chemical bonding and this is described as specific adsorption. Precipitation and co-precipitation both involve the formation of new solid phases in which Cd becomes incorporated, e.g. CdS and Ca-Cd carbonates (McLean and Bedsoe, 1992).

From the literature, it has been shown that Cd interacts with the hydroxyl groups at the surface of metal oxides and short-range ordered aluminosilicates. For the metal oxides, the degree of binding was stronger than electrostatic forces and the mechanism of ion exchange was related to the electronegativity of Cd and O. For silicates, specific adsorption has been observed but other processes such as isomorphous substitution at the time of mineral formation also occur. For example, Cd was specifically adsorbed to kaolinite at lattice imperfections including exposed structural hydroxyl groups at broken edges while the presence of Cd in smectites and vermiculites appears to be predominantly due to isomorphous substitution. Finally, for carbonates, a fast reaction that involves exchange of Ca^{2+} and Cd^{2+} results in the incorporation of Cd into Ca-Cd carbonates (Christensen and Huang, 1999).

In addition to the composition of the soil solids, the distribution of Cd between the aqueous phase and soil solid phases is controlled by many factors such as pH, E_h, the composition of the aqueous phase, e.g. competing heavy metals, presence of dissolved organic and inorganic ligands (Christensen and Huang, 1999). For example, metals such as Cd also interact with natural organic matter and this can result in either its continued presence in the aqueous phase or its transfer to the solid phase soil. For example, Cd may form soluble complexes with low-molecular-weight organic compounds, e.g. fulvic acid, but insoluble complexes with high-molecular-

weight organic components, e.g. humic acid. Colloidal bacteria have enormous surface areas which provide important binding sites for some metal ions in the environment. However there have been different results reported for Cd adsorption to different microbial species in the studies of Huang *et al.* (2005) and Lu *et al.* (2006).

The importance of the form of Cd present in soils especially in relation to contaminated areas will be discussed further in section 1.4.

1.4 Soils Contaminated with Cd from Mining Activities

1.4.1 Case Study I: Cd Contamination in Shipham, Somerset, UK

Thornton (2012) summarised the extent of Cd contamination at Shipham, Somerset, which represents an important case study of the impact of metallifous mining and smelting on the geochemical environment in the UK. From studies carried out in the 1970s, very high concentrations, e.g. 500 mg kg^{-1}, of Cd were found in topsoils. Subsequently, it was found that Shipham sat on an old Zn mining area. Zinc, which was present as smithsonite ($ZnCO_3$), had been mined in shallow pits over the period 1700–1850. The amount of Cd in the ore was appreciable and it was extracted for the manufacture of brass.

A more extensive exploration of the Cd contamination in Shipham was then conducted by the Department of the Environment (DoE) in 1979 (Simms and Morgan, 1988). Approximately 300 samples of garden soils and house dust were collected along with control samples (local background samples) from North Petherton, a nearby Somerset village which had no history of mining. The local background concentrations were <7 mg kg^{-1} and the normal range of Cd concentration in UK soils was 0.1-2.0 mg kg^{-1}. In contrast, the study showed very high concentration of Cd in topsoil (0-5 cm); 90% of the soil samples were over 20

mg kg^{-1} and 60% of samples were over 60 mg kg^{-1} (the highest 340 mg kg^{-1}; the average values in rural area in the UK is 0.39 mg kg^{-1} (0.1-1.8 mg kg^{-1}; Environment Agency, 2007) (Simms and Morgan, 1988). The Cd concentration in house dust averaged 26 mg kg^{-1} (1-373 mg kg^{-1}) with 90% of the samples having values of >10 mg kg^{-1} while the mean concentration for all locations excluding 'hotspots' was 6.9 mg kg^{-1} (<1-8,040 mg kg^{-1}) and for North Petherton was 8 mg kg^{-1} (0.8-150 mg kg^{-1}) (Thornton, 1988). In term of vegetables, 168 samples of garden produce were collected. The Cd concentration in the summer vegetables ranged from 0.02 to 1.77 mg kg^{-1} (mean 0.23 mg kg^{-1}) while in winter vegetables it ranged from 0.01 to 3.56 mg kg^{-1} (mean 0.52 mg kg^{-1}). These higher than expected levels in vegetables led to concerns regarding health risks to the population of Shipham (Thornton, 2012).

A programme of blood and urine sampling and later a collaborative study, based on epidemiological data held by the Small Area Health Statistics Unit at Imperial College Medical School, found no adverse effect of Cd to human health in Shipham (Elliott *et al.*, 2000); there was only a small increase in the urine-β_2 microglobulin level* of some residents. The reasons for the minimal effects on human health were considered to be: (i) the effect of high soil pH (average soil pH 7) which limits Cd availability for plant uptake; (ii) the competitive effect of Zn which was present at higher amounts in both soils and plants; (iii) the small percentage of home grown produce consumed as part of normal diets in the UK; and (iv) access to clean (mains supplied) drinking water and thus low ingestion of Cd via water (Thornton, 2012)

* "β2-microglobulin is a low-molecular-weight protein that forms the light chain component of class I histocompatibility (HLA: human leukocyte antigen) antigens. Increased urine levels are seen in proximal tubular renal damage due to a variety of causes, including: (i) cadmium, mercury, lithium, or aminoglycoside toxicity; (ii) pyelonephritis; and (iii) Balkan nephropathy, a chronic interstitial nephritis of unknown etiology."
(http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/300243).

1.4.2 Case Study II: Cd Concentration in Soils and Rice Grains in Asia

This section will describe the status of Cd-contamination in soils and rice in many countries in Asia, factors controlling the transformation of Cd speciation and the factors affecting Cd bioavailability in paddy field soils.

Over the period 1990-1995, Watanabe *et al.,* (1996) investigated the concentration of Cd in rice from 18 countries (10 in Asia – 8 outside Asia). Rice was sampled at random from locations such as markets, small rice shops and directly from paddy fields. The results showed that, amongst the Asian countries, the lowest national geometric mean[1] was 2.67±1.60 µg kg^{-1} (n=8) for Australia whilst the highest geometric mean of 55.7±2.86 µg kg^{-1} (n=788) was observed for Japan. The geometric mean for Thailand was 15.0±1.43 µg kg^{-1} (n=13). For the non-Asian countries assessed, the lowest geometric mean was 0.85±2.09 µg kg^{-1} (n=3) and the highest 133±3.23 µg kg^{-1} (n=22), for Spain and Colombia, respectively. However, they were all less than the safe level of 0.4 mg kg^{-1} for Cd in rice grains (FAO/WHO, 2011).

During the 2000s, however, many academic papers from Asian countries like China and Thailand reported unsafe levels of Cd in soils and in rice (Table 1.3). This presents a worrying scenario for future human health in these areas based on the effects that were encountered in Japan following the major contamination of the Jinzu River Basin by the Mitsui Mining Company in the early part of the 20th Century. Even in 1974, more than four decades after the issue was first identified, Cd concentrations of <1.00-6.88 and 0.02-1.06 mg kg^{-1} in soils and rice, respectively, were measured (Toyama Prefecture Department of Health, 1976 in Nogawa *et al*., 2004).

[1] A geometric mean and a geometric standard deviation were used because a log normal distribution was assumed for Cd in rice samples (Watanabe *et al.,* 1996)

Further in the Japan case, a 9-year survey reported that significant human renal damage had widely taken place (Nogawa *et al.*, 2004): In 1976, samples of urine were obtained from 596 inhabitants aged 5 years and over in the 9 hamlets of most heavy polluted areas in the Jinzu River basin. In those samples β2-microglobulin-uria, an indicator of the presence of Cd in the human body, was first found at >4 mg L^{-1} in 100% of men and 90.5% of women over the age of 20. Moreover, concentrations increased with increasing age, perhaps reflecting steady bio-accumulation with time. In contrast, for the 419 people in the control group, β2-microglobulin-uria was detected to a much lesser extent and only then in the older age groups, e.g. 6.1% of men and 7.9% of women in their 70s and 4.2% of men in their 60s.

Even after the mining in Kakehashi River basin ceased in 2001, renal dysfunction has still continued to worse. Moreover, renal dysfunction caused by Cd exposure has been shown to be significant in increasing mortality in the Jinzu river basin and the Kakehashi river basin (Nogawa *et al.*, 2004).

A more detailed consideration of the mechanisms by which Cd causes damage to the human body is given in section 1.5.

Table 1.3 The Cd Concentration of Soils and Rice in Selected Asian Countries

Countries	Cd concentration (mg kg^{-1})		Year	Digestion and Analysis Methods
	Soils	Rice		
China				
Chen Zhou[1]	2.72-4.83	0.01-4.43	2007	HNO_3-H_2O_2, Graphite Furnace Atomic Absorption Spectrometer (AAS)
Jiang Xi[2]	6.79		2008	HNO_3-H_2O_2, AAS
Le Chang[3]	2.01-29.68		2006	HCl:HNO_3 , AAS
Korea[4]	0.355	0.049	2008	ICP MS and AAS
Iran[5]		0.12-0.83	2008	Nitric Perchloric Acid based on ASTM standards, flame (AAS)
Thailand[6]	0.5-284	<0.05-7.7	2005	*Aqua Regia* (3:1 HCl:HNO3), AAS
Vietnam[7]	4.7-10.3		2002	Extraction with DDTC/MIBK, AAS

[1] Zhai *et al.,* 2007, [2] Li *et al.,* 2008, [3] Yang *et al.,* 2006 [4] Soyeon Kim *et al.,* 2008, [5] Zazouli *et al.,* 2008 [6] Simmons *et al.,* 2005, [7] Nguyen *et al.,* 2002

* The safe level of Cd in rice grain is 0.4 mg kg^{-1} (FAO/WHO, 2011)

1.5 Impacts of Cd upon Human Health and Derivation of Safety Limits

From a health impact perspective, humans may be exposed to Cd via ingestion of food and water and via inhalation of air and of cigarette smoke. Most Cd entering the human body, however, comes from food, with an average daily consumption being around 35 µg Cd per day (worldwide), of which 2 µg is absorbed (Cox, 1995). For smokers, tobacco is the most important source of cadmium uptake, with ~10% of the Cd content of each cigarette (1-2 µg) commonly inhaled when the cigarette is smoked (WHO, 1992).

A safe intake level of 7 μg Cd per kg body weight per week (i.e. 70 μg Cd per week for a 70 kg individual) was proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2011). In Cd-contaminated areas, for example in Zn-enriched areas in Thailand where rice is the staple food, the estimated weekly intake values of local communities range from 20 to 82 μg Cd per kg which significantly increases the risk of long-term health problems (Simmons *et al.*, 2005).

Following exposure to Cd, the human body tries to protect itself by producing methallothionein, an enzyme in the liver that binds Cd into complexes (with seven metal atoms in each complex) which are then filtered out by the kidney. Thereafter, the Cd is excreted in urine. However, when the body receives an excessive dose of Cd, toxic effects will occur. This is probably due the amount of Cd present exceeding the binding capacity of this enzyme system. A further reason is that many other metals, including in particular essential metals like calcium (Ca), out-compete Cd for complexation by the enzyme. Symptoms of Cd poisoning include damage to the function of lungs and kidneys and a softening of the bones leading to intense pain in the joints (the Itai-Itai disease mentioned above). The most well-known case of Cd poisoning was in Japan, where many people were diagnosed with Itai-Itai disease following high intakes of Cd as a result of pollution from Zn refineries (Cox, 1995). Although the contamination took place almost 100 years ago, in March 2001, 184 people (3 men, 181 women) were confirmed as having Itai-Itai disease and a further 114 people (17 men, 97 women) were suspected to have this disease (Nogawa *et al*., 2004).

The awareness of the effects of Cd intake led researchers and regulatory organizations to derive recommended safety levels for Cd in vegetables, cereals and sea foods such as crustaceans; which should not exceed 0.05-0.2, 0.1 and 2 mg kg^{-1}, respectively (FAO/WHO, 2011). The Cd concentrations in various foodstuffs and calculated Cd exposures based on dietary intake are shown in Table 1.4.

Table 1.4 Cd Concentrations in Foodstuffs and Estimates of Dietary Cd Exposure

Food item	Cd concentration (mg kg^{-1})		Intake level (g d^{-1})	Extreme exposure* (µg d^{-1})	Typical exposure (µg d^{-1})
	Maximum	Typical			
Vegetables, including potatoes	0.1	0.05	250	25	12.5
Cereals, pulses and legume, including rice and wheat grain	0.2	0.05	200	40	10
Fruit	0.05	0.01	150	7.5	1.5
Oilseeds and cocoa beans	1	0.5	1	1	0.5
Meat of cattle, poultry, pigs and sheep	0.1	0.02	150	15	3
Liver of cattle, poultry, pigs and sheep	0.5	0.1	5	2.5	0.5
Kidney of cattle, poultry, pigs and sheep	2	0.5	1	2	0.5
Fish	0.05	0.02	30	1.5	0.6
Crustaceans	2	0.25	3	6	0.75
Total				93.5	30

* For the `extreme' consumer, Cd intake is estimated either by multiplying the typical exposure by 3 or by using the highest Cd levels found in all relevant foods.

From: Satarug *et al.* (2000)

There has been considerable concern about the high accumulation of Cd in the food chain and many research projects have focused on Cd bioavailability, the mechanism of Cd uptake by plants, and ways to minimize its transfer into the food chain, e.g. Makino *et al.* (2006); Yang *et al.* (2006); Akkajit and Tongcumpou (2010).

Many factors that can affect the transfer of Cd from soil to plant have been reported, e.g. soil pH, redox potential (E_h), organic matter content, soil type, nutrient level,

metal ageing and plant varieties. Soil amendments to restrict uptake have also been investigated, including industrial byproducts, muck (black fine-grained organic sediment), silicon, clays and lime as well as Zn and chloride (e.g. Lombi *et al.*, 2003; Kirkham, 2006). These will be discussed in the sections that follow.

1.6 Factors Controlling Cd Bioavailability in Paddy Field Soils

The portion of a soil metal contaminant that can be absorbed by plant roots is defined as its bioavailable fraction. Once metals have crossed the cell membranes, they can be translocated to other parts of plants.

From the reviews of Kirkham (2006), Alloway (1995) and Grant *et al*. (1999) and research by Ma and co-workers (Ma *et al*., 2006; Ma *et al.,* 2013), many factors may affect metal uptake by plants including those relating to:

(i) the soil - the total concentration of the contaminant, the available forms in soil and the soil properties (i.e. pH, E_h, clay content, presence of organic ligands, chloride, Fe/Mn oxides, and competition from other metal ions such as Zn, Co and Ca)

(ii) the plants – some cultivars and species have a greater capacity to absorb the contaminant, including hyperaccumulator plants (e.g. alpine pennycress; *Thlaspi caerulescens*, tobacco and rice).

(iii) soil management – soil properties can be temporarily or seasonally changed by soil management practices. For instance, flooding soils for periods during rice production will cause fluctuations in soil E_h and pH, while adding lime or manure fertilisers could also modify soil pH.

(iv) time since contamination - the bioavailable form of metals in soils can be reduced as a result of contaminant ageing processes.

In one study of paddy field soils in Thailand (Akkajit and Tongcumpou, 2010), it was revealed that ~71% of total Cd was in exchangeable and reducible forms as defined by the first two steps of the BCR soil sequential extraction scheme. This partly explains the high Cd in rice grains sometimes observed in certain parts of Asia, including Thailand (see Chapter 2), but there are further factors that need to be considered. Khaokeaw *et al.* (2008), in a study of paddy soils in the Mae Sot district of Thailand, found that $CdCO_3$ was the main species in dry soil while CdS increased after the soils were submerged in water (a common agricultural practice in the weeks preceding rice planting). Therefore, if this is a reversible and cyclic process upon wetting and drying, the speciation and thus availability of Cd may vary depending upon the redox status and the sulfur content of the soil. In contrast with those soils, ~50% of total Cd in a Chinese paddy soil was present in the residual fraction (as defined by the Tessier sequential extraction scheme, Tessier *et al.* 1979) which contained mainly primary and secondary minerals (Sun *et al.*, 2007). Thus it is likely that source, ageing processes and farming practices might all be important factors in determining Cd availability to plants.

Overall, the two main factors determining Cd bioavailability in paddy fields are usually considered to be pH and E_h and minor factors are contents of organic matter, Fe/Mn oxides, clay minerals, chloride (Cl), as well as plant cultivars, ageing and competition with other heavy metals such as Zn and Mn (Alloway 1995; Kirkham, 2006). As previously discussed, the main factors, pH and E_h, have a great influence on Cd speciation. Acidic conditions and high E_h status in soil are conducive to the presence of aqueous phase species (see E_h-pH diagram in Figure 1.4). Although still largely dependent on mineralogy, soil type and other factors, there is a current consensus that control of pH and E_h is potentially important for decreasing Cd transfer into the food chain.

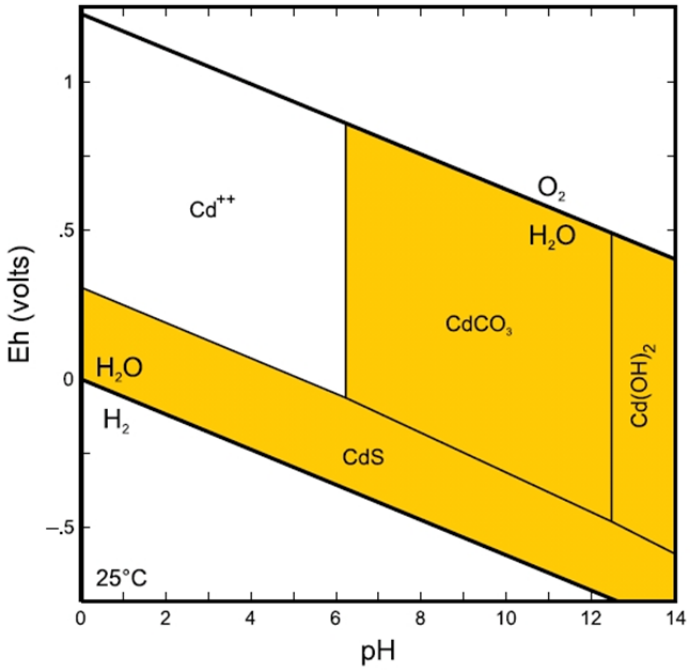


Figure 1.4 E_h-pH Diagram for Cadmium (Wilkin, 2007)

It is important to understand the unique conditions that affect Cd in paddy soils; submergence and drainage are important agricultural practices during the rice-growing season. Submergence of the paddy fields is carried out prior to rice planting whilst water will be drained out during fertilizing and before harvesting. This means that there are rapid changes between anoxic and oxic conditions. In particular the oxidation of soil in the pre-harvest period may result in increased more soluble Cd during the grain-fill phase of rice growth (de Livera *et al.*, 2011).

Some other studies have considered minor factors affecting bioavailability such as iron plaque which generally forms on the root surfaces of rice plants. After Fe and Cd were added to induce different amounts of iron plaque and simulate Cd-polluted soils, Liu *et al.* (2008) found that it was Fe uptake by plants that partly limited the Cd absorption in rice whilst the iron plaque was of little significance.

1.7 Remediation Approaches

Many approaches have been considered and/or attempted for solving the problem of human consumption of Cd-contaminated rice. For instance, it has been suggested that the land use should be changed to non-food production, e.g. bio-energy plants and flowers, in highly contaminated areas of Thailand. Many researchers (e.g.Tokunaga *et al.*, 1996; Kamiya, 2006; Mcgrath and Zhao, 2003) have postulated Cd remediation approaches, e.g. soil washing with chemical solutions (such as $CaCl_2$ and $FeCl_3$) but which has soil nutrient loss and environmental concerns, or physical removal and replacement of topsoil with clean soils which is best suited to small contaminated areas such as those in Japan. Phyto-remediation, using high Cd-accumulating plants such as pennycress and tobacco, has also been suggested. Additional approaches include attempts to transform Cd into unavailable forms by modifying the soil properties, e.g. increasing soil pH, adding organic matter and/or lowering the E_h by water management.

1.8 Summary of Literature

Cd is a rare and toxic heavy metal. It is present at only 0.1-0.15 mg kg^{-1} in the Earth's crust and occurs ubiquitously in rocks and soils. Cd is present at higher concentrations as an impurity in zinc ores, e.g. sphalerite, smithsonite. The main sources of Cd contamination to ecosystems in Europe and Asia were identified as nonferrous mining and the use of phosphate fertilisers. In chemical terms, Cd is a soft Lewis acid and forms complexes with Cl^-, OH^- and ligands in the aqueous phase. In soils, Cd can bind with negative charges on the surfaces of or within the structure of various phases, e.g. metal oxides, aluminosilicates, phyllosilicates, carbonate and organic matter. Adsorption/desorption is the dominant process controlling the distribution of Cd between the aqueous and the solid phase.

As mentioned above, Cd contamination in soils occurred both in Europe and Asia. In particular in Asia, Cd concentration in soils and rice substantial have increased over the last few decades in some areas of rice growing countries, e.g. China (up to ~29 mg kg^{-1} in soils and up to ~4 mg kg^{-1} in rice) and Thailand. Key factors that affected the Cd uptake by plants were soil pH, E_h, plant varieties and farm practices. With regard to this kind of Cd exposed, Cd was mainly intake to human body via food in particular rice as a stable meal. Having high intake of Cd for a long time (>7 μg kg^{-1} body weight per week) posed a risk to human health, e.g. increased likelihood of kidney dis-function and the softening of bones. To remediate the Cd-contaminated soils, many approaches have been introduced such as the change of land use to avoid food chain activities, soil washing, reduced Cd bioavailability in soils and phyto-remediation.

1.9 Research Aims

The overall aim of this project is to characterise the source, pathways and fate of Cd in the soils of the Mae Tao watershed in Thailand, which have become contaminated following mining activity in the nearby mountains (see Chapter 2). The findings from this project will provide missing knowledge about the geochemical transformations affecting: (i) Cd speciation during its transport from the mining area to the paddy fields: and (ii) Cd distribution within a paddy field system which has many check-dams as a key component of the water supply system, i.e. canal and creek. By investigating ways of reducing plant-available Cd, it could also point the way to a promising remediation strategy.

The specific objectives are to:

(i) characterise the mineral form of Cd present in the mining waste and ore material and any transformations in chemical form occurring during riverine transport of the material as suspended particulate matter;

(ii) elucidate the geochemical transformations of Cd within the community irrigation system and upon transfer to the paddy field soils;

(iii) establish links between the solid phase Cd speciation and Cd availability to rice plants by using bioavailability assessment methods e.g. Tessier sequential extraction, BCR extraction and a stable isotope exchange technique;

(iv) trial selected soil amendments in laboratory experiments to reduce Cd availability to rice plants.

References

Akkajit, P. and C. Tongcumpou. (2010) Fractionation of metals in cadmium contaminated soil: relation and effect on bioavailable cadmium. *Geoderma* 156(3-4): 126-132.

Alloway, B.J. (1995) Cadmium. In Alloway, B.J. (Ed.) Heavy metals in soils. Second edition. London: Blackie Academic & Professional. pp. 122-151.

Bernard, P. (2009) Secondary batteries – Nickel Systems, Nickel-Cadmium sealed. Encyclopedia of Electrochemical Power Sources. pp. 459-481.

Calmano, W., J. Hong and U. Förstner. (1993) Binding and mobilization of heavy metals in contaminated sediments affected by pH and redox potential. *Wat. Sci. Tech*. 28(8-9): 223-235.

Chaney, R.L., (2010) Cadmium and zinc. In Hooda, P.S. (Ed.) Trace element in soils. John Willey & Sons, Chichester, UK. pp. 409-439.

Cox, P.A. (1995) The elements on earth: Inorganic chemistry in the environment. Oxford: Oxford University Press.

Davidson, C.M., A.L. Duncan, D. Littlejohn, A.M. Ure and L.M. Garden. (1998) A critical evaluation of the three-stage BCR sequential extraction procedure to assess the potential mobility and toxicity of heavy metals in industrially-contaminated land. *Analytica Chimica Acta* 363(1): 45-55.

de Livera, J., M.J. McLaughlin, G.M. Hettiarachchi, J.K. Kirby and D. G. Beak. (2011) Cadmium solubility in paddy soils: Effects of soil oxidation, metal sulfides and competitive ions. *Science of the Total Environment* 409(8): 1489–1497.

Ďurža, O. (1999) Heavy metals contamination and magnetic susceptibility in soils around metallurgical plant. *Physics and Chemistry of the Earth, Part A: Solid Earth and Geodesy* 24(6): 541-543.

Elliott, P., R. Arnold, S. Cockings, N. Eaton, L. Jarup, J. Jones, M. Quinn, M. Rosato, I. Thornton, E. Tristan and J. Wakefields. (2000) Risk of mortality, cancer incidence and stroke in a population potentially exposed to cadmium. *Occupat. Environ. Med*. 57(2), 94–97.

Environment Agency. (2007) UK Soil and Herbage Pollutant Survey. Report No. 7: Environmental concentrations of heavy metals in UK soil and herbage. Bristol: Environment Agency.

EPA. (1999) Cadmium Geochemistry and K_d Values: Understanding variation in partition coefficient, K_d values volume II: Review of Geochemistry and Available K_d Values for Cadmium, Cesium, Chromium, Lead, Plutonium, Radon, Strontium, Thorium, Tritium (3H), and Uranium. pp. 5.5-5.14.

FAO/WHO (2011) Joint FAO/WHO standards programme codex committee on contaminants in foods. Fifth session. The Hague, The Netherlands, 21 - 25 March 2011.

Fraga, C.G. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular Aspects of Medicine* 26(4-5): 235-244.

Friburg, L., M. Piscator, G.F. Nordberg and T. Kjellström. (1974) Cadmium in the environment. Second edition. Ohio: CRC Press, Inc.

Grant, C.A., L.D. Bailey and M.J. McLaughlin. (1999) Management factors which influence cadmium concentration in crops. In McLaughlin, M.J. Cadmium in soils and plants. Dordrecht: Kluwer Academic Publishers. pp. 151-187.

Greenwood, N and A. Earnshaw. (1997) Chemistry of the elements. Second edition. Oxford: Butterworth-Heinemann.

Handbook of Mineralogy (2005) www.handbookofmineralogy.com/pdfs/greenockite.pdf. Mineralogical Society of America.

Huang, Q., W. Chen and L. Xu. (2005) Adsorption of Copper and Cadmium by Cu- and Cd-Resistant Bacteria and Their Composites with Soil Colloids and Kaolinite. *Geomicrobiology* 22(5): 227-236.

Jung, M.C. and Thornton I. (1996) Heavy metal contamination of soils and plants in the vicinity of a lead-zinc mine, Korea. *Applied Geochemistry* 11(1-2): 53-59.

Kabata-Pendias, A. and A.B. Mukherjee. (2007) Trace Elements from Soil to Human. Berlin: Springer-Verlag.

Kaji, M. (2012). Role of experts and public participation in pollution control: the case of Itai-Itai disease in Japan. *Ethics in Science and Environmental Politics* 12(2), 99-111.

Kirkham, M.B. (2006) Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation and amendments. *Geoderma* 137(1-2): 19-32.

Krishnamurti, G.S.R., P.M. Huang, K.C.J. van Rees, L.M. Kozak, and H.P.W. Rostad. (1995) Speciation of particulate-bound Cadmium of soils and its bioavailability. *The Analyst* 120(3): 659-665.

Liu, H., J. Zhang, P. Christie and F. Zhang. (2008) Influence of iron plaque on uptake and accumulation of Cd by rice (*Oryza sativa L.*) seedlings grown in soil. *Science of the Total Environment* 394(2-3): 361-368.

Lombi, E., R.E. Hamon, S.P. McGrath and M.J. McLaughlin. (2003) Lability of Cd, Cu, and Zn in polluted soils treated with lime, beringite, and red mud and identification of a non-labile colloidal fraction of metals using isotopic techniques. *Environmental Science and Technology* 37(5): 979-984.

Lu, W.B., J.J. Shi, C.H. Wang and J.S. Chang. (2006) Biosorption of lead, copper and cadmium by an indigenous isolate Enterobacter sp. J1 possessing high heavy-metal resistance. *J Hazard Mater* 134(1-3): 80-6.

Ma, Y.B., E. Lombi, I.W. Oliver, A.L. Nolan and M.J. McLaughlin. (2006) Long-term aging of copper added to soils. *Environmental Science & Technology.* 40(20): 6310-6317.

Ma, Y., E. Lombi, M.J. McLaughlin, I.W. Oliver, A.L. Nolan, K. Oorts and E. Smolders. (2013) Aging of nickel added to soils as predicted by soil pH and time. *Chemosphere* 92(8): 962-968.

Makino, T. (2007) Heavy metal pollution of soil and a new approach to its remediation: research experiences in Japan. In 'Proceedings of the International Workshop of ESAFS8: New Solutions to Soil Pollution and Distribution, Bioavailability and Management of Heavy Metals'. (Ed ZS Chen) pp. 55-74 (Food and Fertilizer Technology Center for the Asian and Pacific Region: Taipei, Taiwan).

Makino, T., K. Sugahara, Y. Sakurai, H. Takano, T. Kamiya, K. Sasaki, T. Itou and N. Sekiya. (2006) Remediation of cadmium contamination in paddy soils by washing with chemicals: Selection of washing chemicals. *Environ. Pollut.* 144(1): 2-10.

McGrath, S.P. and F.J. Zhao. (2003) In Kirkham, M.B. 2006. Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation and amendments. *Geoderma* 137(1-2): 19-32.

McLaughlin, M.J., D.R. Parker and J.M. Clarke. (1999) Metals and micronutrients - food safety issues. *Field Crops Research* 60(1-2): 143-163.

McLean, J.E. and B.E. Bedsoe. (1992) The behaviour of trace metals in soil. US EPA Report EPA/540/S-92/018.Mineral Information Institute. (2009) WWW document URL http://www.mii.org/Minerals/photocad.html Accessed 10 December 2009.

Morrow, H. (2010). Cadmium and Cadmium Alloys. *Kirk-Othmer Encyclopedia of Chemical Technology*. John Wiley & Sons. pp. 1–36.

Nogawa, K., E. Kobayashi, Y. Okubo and Y. Suwazono. (2004) Environmental cadmium exposure, adverse effects and preventive measures in Japan. *BioMetals* 17(5): 581-587.

Opila, R.L., J. Church, F. Fang and J.L. Mass. (2012). Surface Analysis Meets Microscopy: Chemical Changes in Matisse Paintings. *Microscopy and Microanalysi*, 18(Supplement S2): 896-897.

Pan, J., J.A. Plant, N. Voulvoulis, C.J. Oates and C. Ihlenfeld. (2010) Cadmium levels in Europe: implications for human health. *Environ Geochem Health* 32(1): 1–12.

Satarug, S., M.R. Haswell-Elkins, and M.R. Moore. (2000) Safe levels of cadmium intake to prevent renal toxicity in human subjects. *British Journal of Nutrition* 84(6): 791-802.

Scoullos, Michael J.; Vonkeman, Gerrit H.; Thornton, Iain; Makuch, Zen (2001). *Mercury, Cadmium, Lead: Handbook for Sustainable Heavy Metals Policy and Regulation*. Springer. ISBN 978-1-4020-0224-3.

Simmons, R.W., A.D. Noble, P. Pongsakul, O. Sukreeyapongse and N. Chinabut. (2008) Analysis of field-moist Cd contaminated paddy soils during rice grain fill allows reliable prediction of grain Cd levels. *Plant and Soil* 302(1-2): 125–137.

Simmons, R.W., P. Pongsakul, D. Saiyasitpanich andS. Klinhoklap. (2005) Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice downstream of a zinc mineralized area in Thailand: implication for public health. *Environmental Geochemistry and Health* 27(5-6): 501-511.

Simms, D.L. and H. Morgan. (1988) Chapter 1: Introduction. *The Science of the Total Environment* 75: 1-10.

Sloan, J.J., R.H. Dowdy, M.S. Dolan and D.R. Linden. (1997). Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils. *Journal of Environmental Quality* 26(4): 966-974.

Swaddiwudhipong, W., P. Limpatanachote, P. Mahasakpan, S. Krintratun, B. Punta and T. Funkhiew. (2011) Progress in cadmium-related health effects in persons with high environmental exposure in northwestern Thailand: A five-year follow-up. *Environmental Research* 112: 194-198.

Stoeppler, M. (1991) II.6 Cadmium. In Merian, E. (Ed.) Metals and their compounds in the environment. Weinheim: VCH Verlagsgesellschaft mbH.

Sun, L., S. Chen, L. Chao and T. Sun. (2007) Effects of flooding on changes in E_h, pH and speciation of cadmium and lead in contaminated soil. *Bull Envi Contam Toxicol* 79(5): 514-518.

Tessier. A., P.G.C. Campbell and M. Bisson. (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* 51(7): 844- 851.

Tokalioglu, S., S. Kartal, and G. Birol. (2003) Application of a three-stage sequential extraction procedure for the determination of extractable metal contents in highway soils. *Turk J Chem* 27: 333-346.

Tokunaga, S. (1996) Soil Pollution: State of the Art in Japan and Soil Washing Process. *Clean Technology* 2(2):126-139.

Tomohito, A., A. Kawasaki, K. Baba, S. Mori and S. Matsumoto. (2009) Effect of water management on cadmium and arsenic accumulation and dimethylarsimic acid concentration in Japanese rice. *Environ. Sci. Technol*. 43(24): 9361-9367.

Thornton, I. (1988) Chapter 3: Metal content of soils and dusts. *The Science of the Total Environment* 75: 21-39.

Thornton, I. (2012) Environmental geochemistry: 40 years research at Imperial College, London, UK. *Applied Geochemistry* 27(5): 939-953.

USGS. (2002) Cadmium by J. Plachy. http://minerals.usgs.gov/minerals/pubs/commodity/cadmium/cadmmyb02.pdf.

USGS. (2013) http://minerals.usgs.gov/minerals/pubs/commodity/cadmium/.

Watanabe, T., S. Shimbo, C.S. Moon, Z.W. Zhang and M. Ikeda. (1996) Cadmium contents in rice samples from various areas in the world. *Science of the Total Environment* 184(3): 191-196.

Welch, R.W. and W.A. Norvell. (1999) Mechanism of cadmium uptake, transportation and deposition of plants. In McLaughlin, M.J. and B.R. Singh. (Eds.) Cadmium in soils and plants. London: Kluwer Academic Publishers. pp. 125-150.

WHO Regional office for Europe. (2000) Chapter 6.3 cadmium. WWW document URL http://www.euro.who.int/document/aiq/6_3cadmium.pdf Accessed 9 November 2009.

WHO. (1992) Cadmium: Environmental Health Criteria 134. http://www.inchem.org/documents/ehc/ehc/ehc134.htm Accessed 9 November 2009.

WHO. (1996) Trace elements in human nutrition and health. Geneva.

WHO. (2008) Guidelines for Drinking-water Quality. Third Edition. Geneva.

Wilkin, R.T. (2007) Cadmium. In R.G. Ford, R.T. Wilkin, and R.W. Puls (eds.) Monitored natural attenuation of inorganic contaminants in ground water volume 2: Assessment for non-radionuclides including arsenic, cadmium, chromium, copper, lead, nickel, nitrate, perchlorate, and selenium. EPA. pp. 1-9.

Worldbank Group. (1998) Pollution prevention and abatement handbook. WWW document URL http://ifcln001.worldbank.org/ifcext/enviro.nsf/AttachmentsByTitle/p_ppah_pguiCadmium/$FILE/HandbookCadmium.pdf Accessed 2 October 2009.

WWW.cadmium.org. (2009) Introduction. WWW document URL http://www.cadmium.org Accessed 9 November 2009.

Yang, Q.W., C.Y. Lan, H.B. Wang, P. Zhuang and W.S. Shu. (2006) Cadmium in soil-rice system and health risk associated with the use of untreated mining wastewater for irrigation in leaching, China. *Agricultural Water Management* 84:147-152.

Zimmerman, A.J., and D.C. Weindorf. (2010) Heavy Metal and Trace Metal Analysis in Soil by Sequential Extraction: A Review of Procedures. *International Journal of Analytical Chemistry* 2010: Article ID 387803, 7 pages.

Chapter 2 Cd Contamination in the Mae Tao Watershed in Northwestern Thailand

2.1 Background Information

2.1.1 Geography, Climate and Land-use

The study area is in Mae Sot district, Tak province, northwestern Thailand, near the Thailand-Myanmar border (Figure 2.1). A major topographical characteristic of this region is the mountain range which lies in the East of the Province and runs from the north to the west of Thailand. The main mountain range (maximum altitude 2,565 m), called Thanonthongchai, consists of various rocks including limestone, mudstone, shale, granite and sandstone, formed during many different periods, from Pre-Cambrian to Tertiary eras. The mountainous area is typically covered with tropical rain forests which are the source of many small creeks, e.g. Mae Tao Creek and Mae Ku Creek. All creeks eventually flow into the Mei River which is an international river running along the border between Thailand and Myanmar. The Mae Tao Creek is one such example: originating in the mountainous area in the east, it descends to the flat plain area in the centre and combines with the Mei River in the west of the province.

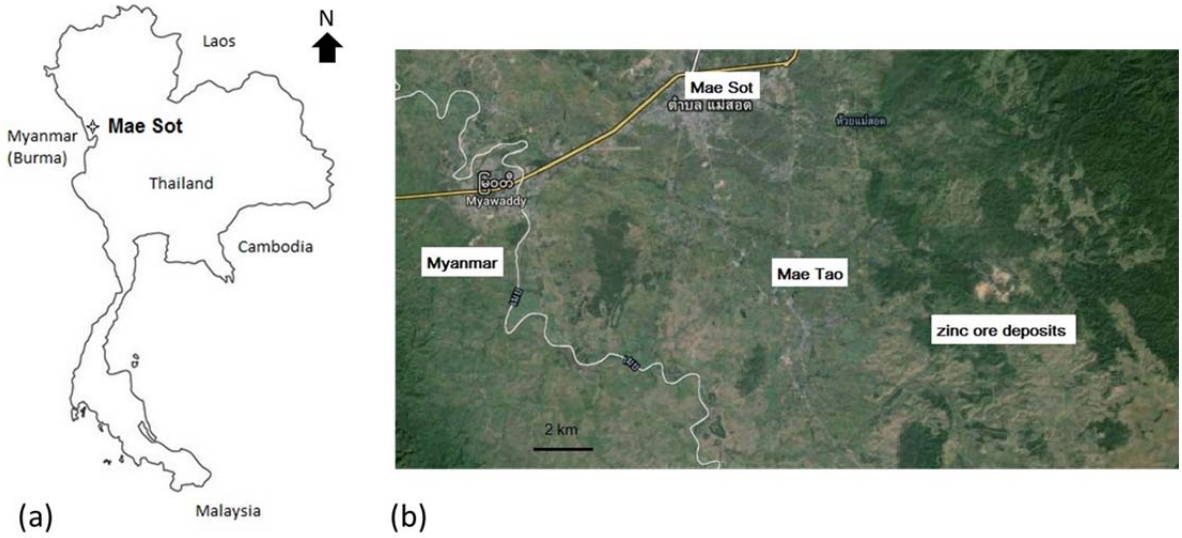


Figure 2.1 (a) Map of Thailand showing Tak province and (b) map of the study site showing Mae Sot and Mae Tao with the mountainous area to the east and the border with Myanmar to the west

The mean annual rainfall is ~1,435 mm. Rainfall is concentrated in the wet season (June – August) when the rainfall is over 800 mm due to the influence of the southwest monsoon from the Indian Ocean and the Andaman Sea. The average humidity is ~75% and the mean annual temperature is 26.4˚C (1977-2008) (Figure 2.2). The influence of the south-west monsoon from the Andaman Sea means that, in comparison with other parts of Thailand, this is one of the most humid areas (Figure 2.2).

With respect to the geology, Paijitprapapon *et al.* (2006) reported that the eastern part of three sub-districts (Phra That Pha Daeng, Mae Tao and Mae Ku) in Mae Sot district, i.e. the mountainous area, was geologically classified as an Upper Triassic Formation (227 to 205 million years old) consisting of conglomerate with pebbles of limestone, chert quartzite, red sandstone, and siltstone. In the central part, the rock formation was identified as Lower Jurassic (195 to 176 million years old), with recent mountainous landscape and abundant shale, argillaceous limestone, sandstone and siltstone. In the western part, unconsolidated and semi-consolidated

layers laid down in the Quaternary (1.8 million years ago to recent) period occur as terraces, colluviums, and alluvial deposits consisting of sand, pebble, clay, white clay, granite soil and red residual soil.

The land-use activities can be divided into three main types: those dominating in the mountainous area, those taking place in the undulating and rolling central area and finally those prevailing in the flat plains in the west.

In the mountainous area, in particular Doi Padaeng (Padaeng mountain), the most important land-use activity is mining. This region has plentiful ore resources such as those containing Pb, Zn, Cu and Cd (Lertlakanawong, 2005; Department of Minerals Resources, 2009; Thai Meteorological Department, 2009). Some 40 years ago, a large deposit of Zn ore was discovered and later exploration confirmed that this is the biggest Zn ore source not only in Thailand but in Southeast Asia. This has led to major ore extraction and processing activities in the mountainous area and two main Zn mining companies, the Padeang Industries Company and the Tak Mining Company, have been operating here over the past four decades.

In the Padaeng deposit, Zn minerals are concentrated in the form of an ore body with a thickness of 220 m at a depth of 30 m below the top soil. The mineral formations comprise both primary and secondary deposits. The primary deposit was formed in the host rock of dolomitic limestone. Here sulfide minerals, including sphalerite (ZnS), galena (PbS) and pyrite (FeS_2), have been identified. The secondary deposit was the result of primary mineral alteration due to weathering processes caused by lowering of the surface and ground waters and chemical reaction of Zn with oxygen and water. The secondary deposit is clearly evident in a colloform band which contained high concentrations of Zn in the compounds of carbonate and silicate minerals such as smithsonite ($ZnCO_3$) and hemimorphite ($Zn_4(Si_2O_7)(OH)_2.2H_2O$) (Paijitprapapon *et al*., 2006).

To the west of the mountainous region is the undulating and rolling area which has slopes of 2-20%. The soils here have a variety of textures but are mainly loams and clays and the soil depth is shallow (25-50 cm) (Table 2.1). The soil permeability depends on the soil texture but the drainage and flow is generally moderate to fast. In summary, the soil chemical properties in these areas do vary but the soils are usually slightly acidic (Table 2.2). Villagers usually grow cash crops such as maize and cassava in these areas (Land Development Department-Thailand, 1990; Lertlakanawong, 2005).

The third and largest area is the flat plains. Here the soil textural class is predominantly clay and the soils are very deep, i.e. greater than 100 cm. The soils were thought to be slightly acidic, show poor permeability and contain low levels of key nutrients, e.g. the organic matter content (both lower and upper soils) is in the range 0.4-2.6% (Table 2.2). However, following an intensive survey in 2005, a research team from the National Center of Excellence for Environmental and Hazardous Waste Management (Chulalongkorn University) reported that the pH of most of the soil samples was near-neutral and almost half of the soil samples had intermediate-to-high organic matter contents (2.5-4.5%) (cf most Thai agricultural soil ranges 1-3%) (Akkajit and Tongcumpou, 2010).

The land-use of the flat plains is mainly paddy fields which are used to grow rice during the wet season and soy bean, red onion and garlic during the dry season. In the rice-growing season, the soils are flooded using water from the Mae Tao Creek via a community irrigation system. The high quality of the rice product is very well-known and this area has twice been awarded the Thai “best rice of the year”. Unfortunately, this is the largest area of Cd contamination of soil and crops because Cd originating from the Zn mining area has entered the creek waters which in turn enter the paddy fields through the community irrigation system. The irrigation system has many branches of canals providing water to all villagers’ paddy fields (Land Development Department-Thailand, 1990; Lertlakanawong, 2005).

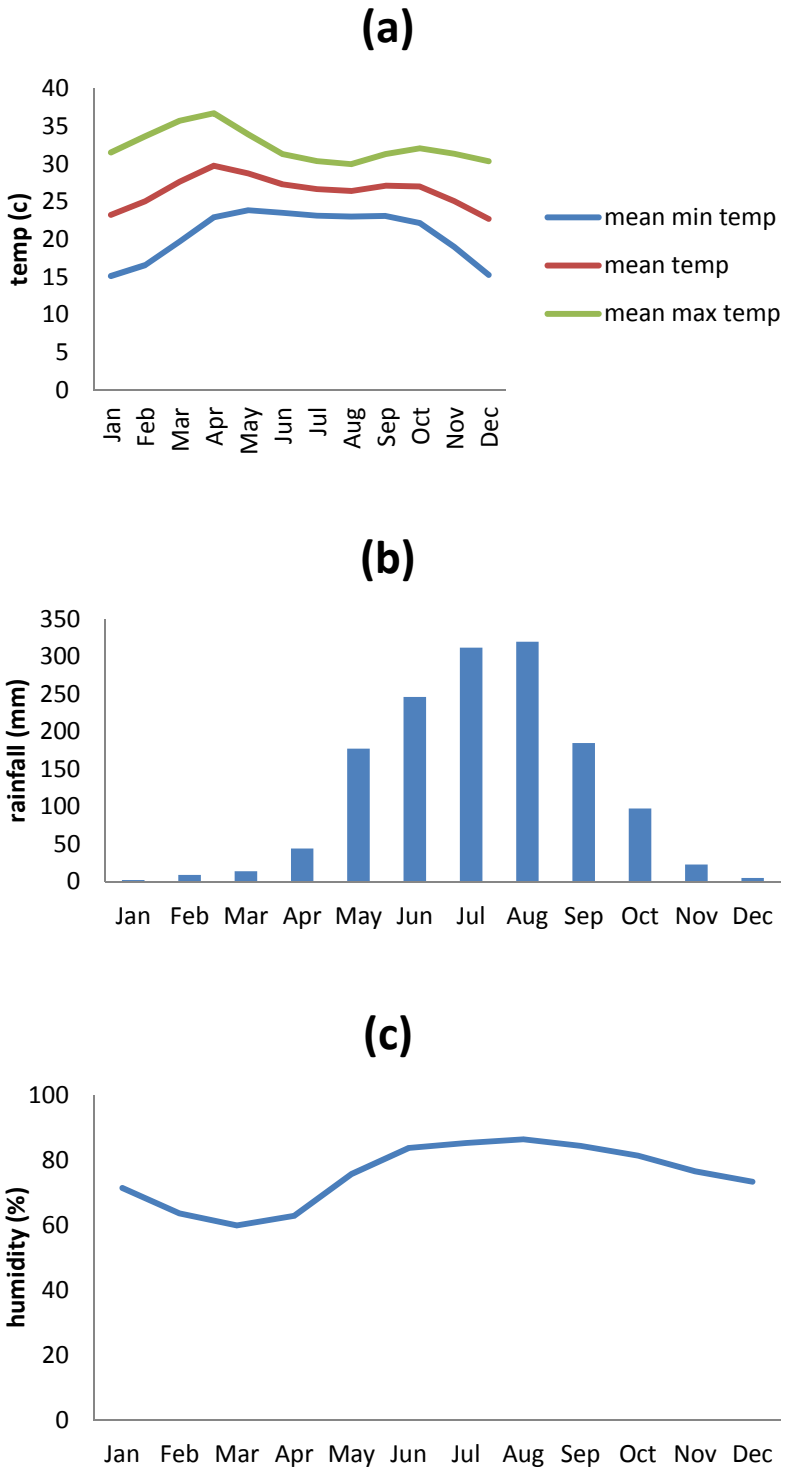


Figure 2.2 Climatic conditions in Mae Sot district, Tak province, Thailand (1977-2008): (a) mean monthly temperature, (b) mean monthly rainfall and (c) mean monthly humidity

From Thai Meteorological Department (2009)

Table 2.1 Physical Properties of Soils in Tak Province, Thailand

Category (slope)		Texture (upper soil)	Depth[1]	Permeability[2]	Drainage	Surface run off
1 (0-2%)	Flat	Clay, Silty clay, Loam clay, Loam, Sandy loam	Very deep	Low, moderately low	Slow, moderately slow	Slow
2 (2 – 8%)	Flat – Undulating and rolling	Clay, Silty clay, Silty loam, Clay loam	Medium deep – deep	Moderate – rapid	Moderate	Moderate – slow
	Slightly undulating – Undulating	Clay loam + pebbles	Shallow – very shallow	Low-moderate	Moderately low	Moderate – fast, moderate
3 (2 – 20%)	Undulating and rolling	Clay loam + pebbles, Loam + slit, Loam	Shallow	Rapid	Well-drained	Fast, moderate - fast
4 (>35%)	Mountainous area	Depends on the parent rock	Uncertainty	-	Moderately well-drained	Fast

From Land Development Department-Thailand (1990)

[1] Very shallow (<25cm) , shallow (25-50 cm), medium (50-100 cm), deep (100-150 cm) and very deep (>150 cm)
[2] Very Slow (<0.125 cm hr^{-1}), slow (0.125-0.5 cm hr^{-1}), Slightly slow (0.5-2.0 cm hr^{-1}), moderate (2.0-6.25 cm hr^{-1}), slightly fast (6.25-12.5 cm hr^{-1}), fast (12.5-25.0 cm hr^{-1}) and very fast (>25 cm hr^{-1}) (O'Neal, 1952 in Wiwatwongwana, 2003)

Table 2.2 Chemical Properties of the Soils in Tak Province, Thailand

Category (slope)	Soil depth	Organic matter (%)	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})	pH
1 (0-2%)	Upper	0.4 – 2.6	2.2 – 19.9	25.0 – 147	5.5 – 7.0
	Lower	1.3 – 2.0	4.5 – 16.4	39.1 – 140	4.5 – 8.0
2 (2–8%)	Upper	1.4 – 8.0	3.2 – 29.7	27.6 – 209	5.0 – 8.0
	Lower	1.7 – 6.9	3.4 – 22.9	21.6 – 167	4.5 – 8.0
3 (3–20%)	Upper	1.4	2.9	65.6	5.5 - 6.5
	Lower	1.2	2.1	60.4	4.5 – 5.5
4 (>35%)	Upper	-	-	-	-
	Lower	-	-	-	-

From Land Development Department-Thailand (1990)

2.1.2 Villagers and Agricultural Practices

The communities in cadmium-contaminated areas live in rural agricultural villages. These small villages consist of approximately 500 households and are scattered over three sub-districts (Phra That Pha Daeng, Mao Tao and Mao Ku) of Mae Sot (Table 2.3 and 2.4). Most villagers migrated from the north of Thailand about 100 years ago and the Karen ethnic group, the original villagers, moved up to the most elevated area (Baan Pha Deh). Even now, their lifestyle and traditions are the same as the highland Thai people, e.g. ceremonies relating to the Buddhist religion and to other spiritual matters. Moreover, they also introduced their local wisdom relating to the community irrigation system (CIS), into these areas (Lertlakanawong, 2007).

Rain-fed rice farming with beans as a secondary crop versus cash crops are the two kinds of cultivation practices in this area. In the lowland area, rice farming starts when the rains come in May or June and finishes in December every year. After that the villagers grow beans such as soy bean or garlic or shallot in the dry season (January–April). In the undulating and rolling areas, the farmers raise cash crops

such as maize and cassava which can produce many crops per year (Lertlakanawong, 2005).

The lowland and undulating areas were considered to be most suitable for agriculture because of the abundance of water associated with the high rainfall and with the many water sources from the mountains in the east. The CIS is the main water management system and it enables water from the creeks and irrigation canals to be uplifted with high efficiency and then allocated via irrigation ditches to all community members' fields. In addition, each CIS group has its own rules and an agreement with the other CIS groups who share the same creek.

The CIS consists of a number of check-dams and irrigation ditches. For Mao Tao (a single sub-district) alone, there are 16 check-dams within the CIS (Lertlakanawong, 2005; Phrammathep, 2007). Check-dams are made of available materials such as wood chips, bamboo, rocks, soils and woven bamboo cane enclosing gravel. These dams provide a blockage across the creek and are used to raise the level of the water and to divert it into the irrigation ditches (Figure 2.3). The main irrigation ditch is a water canal connecting to the check dam and discharging to the sub-irrigation ditch and the fields at the end. The main irrigation ditch also has a small gate, made of bamboo, uplifting the water again into sub-irrigation ditches or in-field irrigation channels and then letting the water run into the field at a small outlet (Phrammathep, 2007).

Figure 2.3 The check-dam in a canal uplifting the water level and diverting it to another canal

The CIS is managed by forming an irrigation group which is led by the head of the group with input from all members. The leader must have a reputation of justice and a proven ability to make a fair allocation to members. Furthermore, there are rules and regulations, called a "Communities Irrigation Agreement", which cover agreement, punishment, ceremonies, annual restoration and fees collection (Phrammathep, 2007).

Table 2.3 Number of Villages and Area of Land in Three Sub-districts of Mae Sot

Sub-districts	Number of villages	Area of land (km^2)
Mae Tao	6	45.5
Phra That Pha Deang	7	68.0
Mae Ku	10	163.7
Total	23	277.2

From Lertlakanawong (2005)

Table 2.4 Households and Populations in the Three Sub-districts of Mae Sot

Sub-districts	Number of households	Population		
		male	female	total
Mae Tao (2008)	2,352	3,402	3,127	6,529
Phra That Pha Daeng (2007)	2,004	2,990	2,952	5,945
Mae Ku (2007)	2,788	5,264	4,963	10,227
Total	7,144	11,656	11,042	22,701

From www.thaitambon.com (2009)

2.1.3 Chronology of Cadmium-related Research in Mae Sot, Tak Province, Western Thailand

The official report (Padungtod, 2006) of the International Water Management Institute (IWMI) and Department of Agriculture (DOA), Thailand, regarding the Cd-contamination in Mae Tao watershed led to full environmental investigations, including the health of the local population, carried out by Thai government departments and university researchers, covering the entire lowland area of Mae Sot. The status of the contamination was of great concern because of the high levels of Cd that had been reported in paddy topsoil and creek sediments and because the contamination was wide spread over the lowland area.

The paragraphs which follow give a detailed chronological account of the investigations into Cd contamination in Mae Sot.

1999

Somboon (1999) found that the total Cd and Zn concentrations in soils in the upstream area (4 sites) of Mae Tao watershed were significantly lower than in the downstream area (26 sites; Table 2.5; Figure 2.4). He claimed that the Zn mining

activities operating in the headwater areas of Mao Tao Creek released Cd and Zn into the creek waters leading to contamination of the lowland area.

Table 2.5 Cd and Zn Concentrations in Soils in the Upstream, Downstream and Adjacent Areas of the Mae Tao Watershed (Somboon, 1999)

Soil in Mae Tao watershed	Cd (mg kg^{-1})	Zn (mg kg^{-1})
The upstream area (4 sites)	0.93 (0.10-0.98)	37.9 (30.2-45.0)
The downstream area (26 sites)	50.8 (0.35-300)	1,909 (29.6-11,284)
The adjacent area (outside the watershed, 9 sites)	1.09 (0.48-1.78)	42.1 (26.6-62.4)

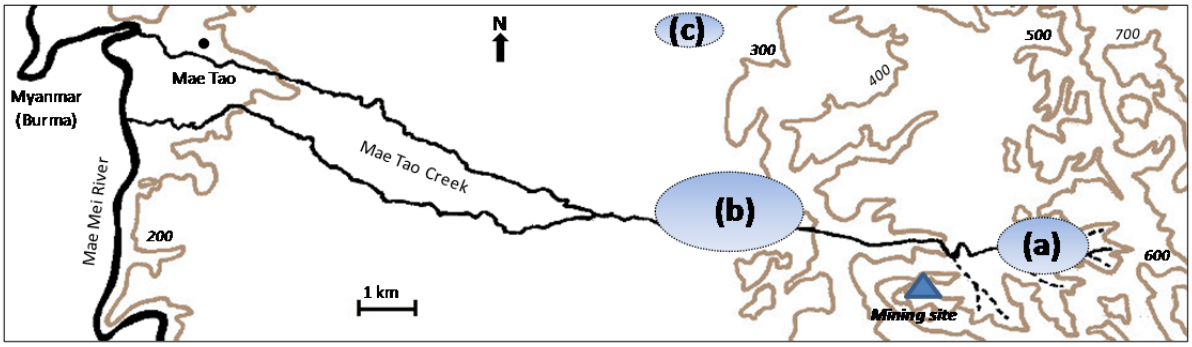


Figure 2.4 a map showing the sampling areas; (a) the upstream area (b) the downstream area and (c) the adjacent area; contours (brown lines) showing altitude in meters

2000-2002

As part of an investigation by the IWMI and the Department of Agriculture, Thailand, to assess the effect of Cd contamination on the environment in Mae Sot district, 578 soil samples and 524 rice grain samples in Baan Pha Deh (village) and Phra That Pha Daeng sub-district were collected. The results showed the severe effects of Cd and Zn contamination in agriculture soils, rice-based crops and on the health of local people. The paddy soils were contaminated with high levels of Cd,

ranging from 0.5–284 mg kg^{-1}, and also of Zn, ranging from 197 – 8,036 mg kg^{-1}. From vertical soil profile measurements, ~89% of total soil Cd and ~83% of total soil Zn were found in the top soil (0–30 cm depth). The results of this study also indicated that these heavy metals were transported to the paddy fields via the waterways and irrigation canals. The Cd and Zn concentrations were the greatest in the fields which directly received water from the Mae Tao Creek. Indeed, the concentration of heavy metals decreased to near background values according to the results for a transect inland from the Mae Tao Creek across the first three fields (~30-50 m).

From the analysis of foodstuffs, it was clear that Cd was absorbed into both rice grains (< 0.05–7.7 mg kg^{-1}) and soy beans (0.34–2.58 mg kg^{-1}) (Table 2.6). Over 90% of the rice grain samples contained Cd at concentrations exceeding the mean typical Thai total Cd in whole grain rice of 0.043±0.019 mg kg^{-1} (0.002-0.156 mg kg^{-1}; surveyed in 1998-1999 and reported by Parkpain *et al.*, 2004) and the Codex Committee on Food Additives and Contaminants (CCFAC) draft Maximum Permissible Level for rice grain of 0.2 mg kg^{-1}. In terms of implications for public health, estimated weekly intake (WI) values were from 20 to 82 µg Cd per kg body weight which presents a significant risk to the local communities' health in the long term (Simmons *et al*., 2005).

Table 2.6 Concentrations of Cd and Zn in Paddy Fields and Rice Grains in Phra That Pha Daeng Sub-district (2000-2002) and Comparison with Background and Maximum Permissible Concentrations

	Cd (mg kg^{-1})	**Background and Maximum Permissible Values (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Background and Maximum Permissible Values (mg kg^{-1})**
soil	0.5-284	0.002–0.141[1] 1.0–3.0[2]	197–8,036	0.1 - 14[1] 300[2]
rice grain	< 0.05–7.7	0.002-0.156[3] 0.2[4]	-	-

[1]The mean "background" concentrations for total Cd and Zn in Thai soils (Pongsakul and Attajarusit, 1999)

[2]The European Union (EU) Maximum Permissible (MP) Cd and Zn concentrations for sludge-amended soils

[3]The mean "background" total Cd concentrations in Thai rice grains (Pongsakul and Attajarusit, 1999))

[4] The 34th Session Codex Committee on Food Additives and Contaminants (CCFAC) (Rotterdam, The Netherlands during the 11th–15th March 2002) proposed a draft provisional Maximum Level (ML) for Cd in rice grain of 0.2 mg kg^{-1} (Codex Alimentarius Commission, 2002)

2004

Following on from the IWMI investigation in 2000-2002, environmental monitoring and quality assessment for Cd contamination in the Mae Tao watershed area was conducted under the auspices of a special technical task force led by the Department of Pollution Control, Ministry of Natural Resources and Environment. The task force reported in April, 2004 and their findings were summarised by Padungtod (2006) as follows:

- The Department of Ground Water Resources found that all groundwater and surface water samples contained Cd at concentrations of <0.001 mg L^{-1} which was considered safe for drinking according to the EU and WHO standards of 0.005 mg L^{-1} and 0.003 mg L^{-1}, respectively.
- The Department of Water Resources reported that all samples along the entire length of the creek contained Cd concentrations between 0.00281 and 0.01 mg L^{-1} which was also considered safe for consumers (<0.005 mg L^{-1} at water hardness >100 mg L^{-1} for the surface water quality standards; Pollution Control Department, Thailand, 1994).
- The Department of Fisheries found that all fish samples had Cd concentrations lower than the safe limit for consumption of < 2 mg kg^{-1}.
- The Department of Mineral Resources found that 88% of sediment samples from Mae Tao Creek contained elevated concentrations of Cd. The highest level (326 mg kg^{-1} soil) was 93 times higher than the lowest concentration.
- Department of Pollution Control reported that 86% of soil samples were contaminated, with Cd concentrations ranging from 61 – 207 mg kg^{-1} soil.
- Rice samples taken from household storage were found to contain Cd from trace levels to 5 mg kg^{-1} rice with an average of 1.33 mg kg^{-1} rice. 91% of rice samples exceeded the Codex Committee on Food Additives and Contaminants (CCFAC) threshold value of 0.2 mg Cd kg^{-1} rice (in 2000).

2005

Chanthachat *et al*. (2005) determined the concentrations of heavy metals in soils from 12 locations along Mae Tao Creek in November 2004 by neutron activation analysis. High concentrations of Cd and Zn were found in 2 locations, specifically Mae Ku Nua Village No.I and the junction between Pha Deh and Mae Tao Mai Village. Cadmium concentrations were 53 and 42 mg kg^{-1} and Zn concentrations were 1,652 and 1,215 mg kg^{-1}, respectively.

In phase II of the project "Plantation Management of the Cd Contaminated Area: Case Study Mae Sot, Tak Province, Thailand (2004-2009)", Tongcumpou *et al*. (2008) classified and zoned the level of Cd concentrations in soil in Mae Tao and Mae Ku sub-catchments (Table 2.7; Figure 2.5). This covered the area of the three sub-districts, Phra That Pha Daeng, Mae Tao and Mae Ku. The results showed that 38.30 km^2 (33.9% total area) were affected by Cd contamination (>3 mg kg^{-1}) and 0.63 km^2 (0.6% total area), mostly along the creeks, had the highest levels of Cd contamination (>60 mg kg^{-1}).

Table 2.7 Zones Based on the Extent of Cd Concentration in Paddy Soils from Phra That Pha Daeng, Mae Tao and Mae Ku (Tongcumpou *et al.*, 2008)

Zone of contamination	Cd concentration (mg kg^{-1})	Area (km^2)	% Total area
No contamination	<3	74.85	66.1
Low contamination	3 – 20	35.04	30.9
Medium contamination	20 – 60	2.73	2.4
High contamination	> 60	0.63	0.6
Total		113.25	100.0

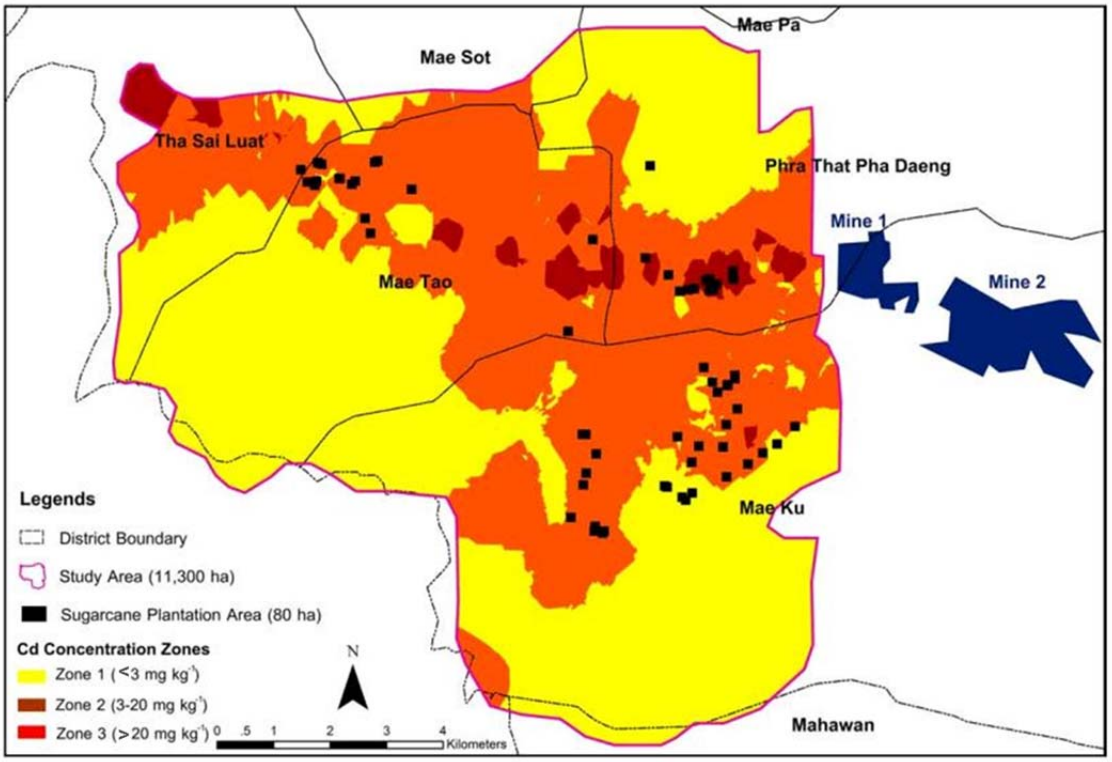


Figure 2.5 Zoning of Cd contamination in the study area and the location of mines (Akkajit and Tongcumpou, 2010)

A further research project focused in particular on differentiating between the forms of Cd in soils. For the narrow range (93% between pH 6.5-7.5) of soil pH for all soil samples in Mae Tao and Mae Ku watershed, Akkajit and Tongcumpou (2010) reported that the mobility of selected metals decreased in the order Cd>Mn>Zn>Pb>Cu>Fe. This was based on the amount of these metals extracted in the first fraction (exchangeable fraction: BCR1) obtained using the European Communities, Community Bureau of Reference (BCR) method. Moreover they confirmed that Cd, Zn and Pb had a strong affinity for Fe/Mn oxides because a large amount of these three metals was extracted in the BCR2 fraction along with Mn and Fe. Interestingly, the well-known factors affecting Cd speciation in soils which are pH, organic matter content and redox potential, showed little correlation with Cd concentration in the BCR2 fraction in all cases (r=0.206, 0.218 and 0.051 respectively). However, in the >20 mg kg^{-1} Cd contamination zone (Figure 2.5),

redox potential showed a strong correlation with the concentration of Cd extracted in BCR1 (r=0.810).

2004

According to IWMI investigation in 2003, physical check-ups on the health of Cd-exposed villagers in 2004 conducted by Mae Sot General Hospital in collaboration with the Bureau of Occupational and Environmental Diseases, Ministry of Public Health, revealed that only half of the local villagers had a safe level of Cd (<2 μg g^{-1} creatinine*) in their urine. The health monitoring of local residents was carried out for 12 villages in the 3 sub-districts of Phra That Pha Daeng, Mae Tao and Mae Ku. The health survey monitored 7,697 people of whom 45.6% had urinary Cd levels <2 μg g^{-1} creatinine. Around 4.9% of the local people had urinary Cd levels between 5-10 μg g^{-1} creatinine and about 2.3% of them had more than 10 μg g^{-1} creatinine. In addition, the urinary Cd levels were also higher among women than men and gradually increased with age. Smokers were more likely to have higher urinary Cd levels than non-smokers. Meanwhile, those who consumed locally grown rice in contaminated areas also had higher urinary Cd levels than those who did not eat local rice products (Swadddiwudhipong, 2007).

2009

Following the screening assessment in 2004, there was a study of the prevalence of renal dysfunction in the high Cd-exposed group (>5 μg g^{-1} creatinine) which comprised 527 people. β2–microglobulin was used as an early indicator of renal tubular dysfunction and serum creatinine and Glomerular Filtration Rate (GFR) were

* "Creatinine is a chemical waste product in the blood that passes through the kidneys to be filtered and eliminated in urine." Normally, concentrations of solutes in urine vary with several biological factors (including the time since last voiding and the volume consumed over the last few over hours); as a result, solute concentrations should be normalized against another characteristics of urine represents changes in solute concentrations. Thus creatinine has been used to adjust or correct the proteins and Cd concentrations in urine.
(http://www.davita.com/kidney-disease/overview/symptoms-and-diagnosis/what-is-creatinine?/e/4726; code of Federal regulations 29,revised as of 1, 2010)

the biomarkers for indicating the severity of chronic disease. The results showed that one-fifth of the study group faced the early stages of kidney disorders because they excreted β2–microglobulin > 1,000 μg g^{-1} creatinine*. Furthermore, it was found that 16.9% of target group had a GFR <60 ml min^{-1} which showed they were in stages 3-5 of chronic kidney disease. Both the increased serum creatinine and decreased GFR seemed to be linked to increased urinary Cd but the correlation were not statistically significant (Limpatanachote, 2007).

Chaiwong *et al.* (2009) reported that 64.3% of 849 children in the Cd affected sub-districts in Mae Sot district had urinary Cd containing <1 μg g^{-1} creatinine. There was no significant difference in the urinary Cd concentration across gender or age groups. However, the mean of urinary Cd concentrations for girls was slightly higher than that for boys (0.129 and 0.125 μg g^{-1} creatinine, respectively). In addition, children of 13-15 years age had a slightly higher level (not statistically significant) of urinary Cd than children of 9-12 years age (0.135 and 0.119 μg g^{-1} creatinine, respectively).

2.2 Summary

The sampling area is part of the Mae Tao district, Tak province, NW Thailand. Mae Tao village, located in the lowlands of the province, which comprises 45.5 km^2 of the total area of the three sub-districts; Phra That Pha Daeng, Mae Tao and Mae Ku (277.2 km^2). The land use in this area is predominantly paddy fields which are used to grow rice in the rainy season and a variety of other crops, e.g. soy bean, during the dry period. Most of the lowland areas, have, however, been contaminated with Cd from the Zn mining activities occurring in the eastern mountains over the past four decades. The Cd contamination in the downstream areas of Mae Tao watershed was discovered in the 1990s but the local population was not informed (e.g. the study of

* There is reversible tubular impairment at β2-microglobulin-uria levels of 1,000 μg g^{-1} creatinine but greater levels lead to irreversible effects (Teeyakasem *et al.*, 2007).

Somboon, 1999) until 2002, when the IWMI reported to the press very high Cd level in soils and rice (0.5 to 284 mg kg^{-1} and <0.05-7.7 mg kg^{-1}, respectively). After that, intensive surveys of Cd contamination in the whole watershed by government agencies were launched to determine the extent of Cd contamination in the lowland area of the watershed. ~34% of the paddy field areas had soil Cd concentrations of (>3 mg kg^{-1}). They also found high concentrations of Cd in affected sediments, soil and rice, in agreement with the IWMI study (up to 326 mg kg^{-1}, 61-207 mg kg^{-1} and trace to 5 mg kg^{-1}, respectively). Low levels of Cd contamination were found in surface and ground water and fish (<0.001 mg L^{-1}, <0.01 mg L^{-1} and <2 mg kg^{-1}, respectively). In contrast with the concentrations found in rice, these levels did not pose a threat to human health.

A zoning map of Cd contamination in Mae Tao watershed showed that the high and medium Cd contaminated areas occurred closed to the creek and canals, supporting the hypothesis that the waterways were instrumental in transferring Cd from the mining area to the paddy fields. Further work then focused on human health. About 7,700 local people were tested and it was found that ~45% had urinary Cd higher than unexposed people (>2 μg g^{-1} creatinine). Moreover, one-fifth of the 527 people who had high urine Cd (>5 μg g^{-1} creatinine) showed symptoms of the early stages of kidney disorders.

Mao Tao watershed is the only watershed in Thailand that has been severely contaminated with Cd originating from only one point source, the Zn-enriched areas in the western mountainous part of the watershed. Furthermore, Cd has been contaminating the creek, canals and rice fields for a period of approximately 40 years. Consequently, health effects in the local population, for whom rice is a staple food, are clearly discernible. For these reasons, the Mao Tao watershed suited the purpose of this project which is the study of cadmium geochemistry and its distribution, in particular in a paddy field system, creek-canal, check-dams, and

fields to gain improved understanding about Cd behaviour. The specific details of the study site will be reported in Chapter 3.

References

Akkajita, P. and C. Tongcumpou. (2010) Fractionation of metals in cadmium contaminated soil: Relation and effect on bioavailable cadmium. *Geoderma* 156(3-4): 126-132.

Chaiwong, S., S. Sthinnopkao, K.W. Kim, S. Chuenchoojit, K. Poopatpiboon, C. Poodendean and W. Supanpaiboon. (2009) Urinary cadmium level in children between nine to fifteen years old in three sub-districts of Tak province in Thailand. *Physics Procedia* 2: 121-125.

Chanthachat, W., A. Busamongkol, S. Khuntong, R. Peripart and S. Loaharojanapanal. (2005) Analysis of heavy metals in soil along Mae Tao river, Tak province by nuclear technique. *Paper presented at 31st Congress on Science and technology of Thailand at Suranaree University of Technology, 18-20 October 2005*.

Codex Alimentarius Commission. 2002 Report of the 34th Session of the Codex Committee on Food Additives and Contaminants, Rotterdam, The Netherlands 11–15 March 2002

Land Development Department,Thailand. (1990) Land-use using for economic crops,Tak 1990. Land Survey and Classify Division, Land Development Department, Minister of Agriculture and Cooperatives. (in Thai)

Lertlakanawong, P. (ed.) (2005) *Summary report: Participatory rural appraisal on Mae Tao sub watershed program*. Bangkok: Local Development Institute. (in Thai). pp.1-50.

Lertlakanawong, P. (ed.) 2007. *Mechanism development for environmental management to geological resources development, Tak Province*. Bangkok: National Health Foundation. (in Thai) pp.1-50.

Limpatanachote, P., W. Swadddiwudhipong, P. Mahasakpan and S. Krintratun. (2007) Cadmium-exposed population in Mae Sot district, Tak province: 2. Prevalence of renal dysfunction in the adults. *J Med Assoc Thai* 92(10): 1345-1353.

O'Neal, A.M. (1952) A key for evaluating soil permeability by means of certain field clues. *Soil Sci. Soc. Amer. Proc.* 16: 312-315 in Pusit Wiwatwongwana, P. (2003) Assessment of soil resources for engineering uses in irrigable areas of Thailand. Land Development Department, Thailand. pp. 4.

Padungtod, C., W. Swaddiwudhipong, M. Nishijo, W. Ruangyuttikarn and T. Inud. (2006) Paper presented at "The fifth session of the Intergovernmental Forum on Chemical Safety (Forum V)", 5 - 29 September 2006. WWW document URL http://www.who.int/ifcs/documents/forums/forum5/thai_padungtod.pdf Accessed 28 October 2009.

Paijitprapapon, A., K. Udomtanateera, O. Udomtanateera, V. Chariyapisuthi and T. Chengsuksawat. (2006) Environmental investigation for causality and remediation of cadmium contaminated soils in Tak province, Thailand. Paper presented in the 7th International Symposium on Environmental Geochemistry: 7th ISEG 24th -27th September 2006 at Beijing international convention center, Beijing, China.

Phrammathep, N. (2007) Lanna community irrigation system. (in Thai) WWW document URL http://gotoknow.org/blog/nhanphromma/139226 Accessed 2 October 2009.

Pollution Control Department, Thailand. (1994). Notification of the National Environment Board No.8 B.E. 2537 (1994) issued under the National Environmental Quality Act B.E. 2535 (1992).

Pongsakul, P and S. Attajarusit (1999) Assessment of heavy metal contaminations in soils. *Thai Journal of Soils and Fertilizers* 21(2): 71–82.

Preeda P., P. Saengthong and P. Pongsakul. (2004) Heavy metal contaminations in relevant to soils and staple crops, Thailand. *Thai Journal of Soils and Fertilizers* 26(1): 31-36.

Simmons, R.W., A.D. Noble, P. Pongsakul, O. Sukreeyapongse and N. Chinabut. (2009) Cadmium-Hazard mapping using a general linear regression model (Irr-Cad) for rapid risk assessment. *Environ Geochem Health* 31(1): 71-79.

Simmons, R.W., P. Pongsakul, D. Saiyasitpanich and S. Klinhoklap. (2005) Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice downstream of a zinc mineralized area in Thailand: Implication for public health. *Environmental Geochemistry and Health* 27(5-6): 501-511.

Somboon, P. (1999) *Distribution of cadmium and zinc in soil from zinc mining activity: A case study of zinc mine, Mae Sot district, Tak province*. Thesis of master degree. Mahidol university, Thailand. (in Thai)

Swadddiwudhipong, W., P. Limpatanachote, P. Mahasakpan, S. Krintratun and C. Padungtod. (2007) Cadmium-exposed population in Mae Sot district, Tak province: 1. Prevalence of high urinary cadmium levels in the adults. *J Med Assoc Thai* 90(1): 143-148.

Teeyakasem W, M. Nishijo, R. Honda, S. Satarug, W. Swaddiwudhipong and W. Ruangyuttikarn. (2007) Monitoring of cadmium toxicity in a Thai population with high-level environmental exposure. *Toxicol Lett.* 169(3): 185–195.

Tongcumpou, C. (2008). *Plantation management on Cd contaminated area: Case study Mae Sot,Tak province, Thailand*. WWW document URL http://home.biotec.or.th/NewsCenter/my_documents/my_files/E8836_Biotech-ISSCAS-Feb08.pdf Accessed 28 October 2009.

www.thaitambon.com. (2009) WWW document URL http://www.thaitambon.com/tambon/tamplist.asp?ID=63 Accessed 9 November 2009.

3 Distribution and Associations of Cd in Paddy Soil Systems in Mae Sot, Thailand*

From the literature presented in Chapter 2, it is clear that the Cd-contamination in Mae Tao watershed is a consequence of point source releases from the zinc mining activities in the zinc-enriched areas (Padaeng deposit) upstream of Mae Tao Creek. The primary and secondary zinc ores e.g. hemimorphite (ZnS) and smithsonite ($ZnCO_3$), respectively, are the minerals used for production of zinc metal in this area. Cd is commonly found as an impurity in those Zn ores. The mining activities generated Cd- and Zn-containing solid waste materials from which Cd and Zn were transferred by the action of rainwater, especially during the rainy season, into the Mae Tao Creek either in aqueous solution or in association with fine particulate matter. Following concerns about Cd-contamination in the paddy fields downstream, the mining company which continues to operate in this area, the Padaeng Company, adopted more stringent procedures to protect the environment. For example, since 2004 they have monitored water emissions from the mine activities and confirmed that Cd concentrations are below the safe limit of 0.03 mg L^{-1}, even during times of heavy rainfall. As described in Chapter 2, recent studies have shown that the Cd concentrations in creek waters are now very low but have determined that concentrations in the creek and canal sediments were elevated. Thus it is hypothesised that contaminated sediment, transported via the network of waterways, is the current source of the Cd being transferred onto the paddy fields. Elevated concentrations of Cd have been found in soils over wide areas of the lowlands but the highest Cd concentrations were found near the waterways. Cadmium concentration greater than the safe level of 0.2 mg kg^{-1}, were also found in rice grains. This is consistent with the observation of high creatinine concentrations in many of the local villagers' urine samples and reports that some villagers were in the early stages of

*Most of the work in this chapter has been published in Kosolsaksakul *et al.* (2014), Environmental Pollution. The co-authors were my supervisors, Dr. Margaret C. Graham, Prof. John G. Farmer and Dr Ian W. Oliver. Dr. Graham was the corresponding author and all three supervisors provided feedback on drafts of the paper.

chronic kidney disease (Padungtod *et al*., 2006; Swadddiwudhipong 2007). This chapter will describe and discuss the results relating to the following objectives: (i) to characterise the geochemical transformations of Cd as it is transported from the main irrigation creek through the system of irrigation canals and within the paddy fields of the Mae Tao watershed; (ii) to use sequential chemical extraction methods to assess Cd associations in soil and its availability to rice plants; (iii) to investigate the relationship between soil-plant transfer factors and the geochemical associations of Cd contaminated paddy soils.

3.1 Methods

3.1.1 Study Location

Mae Tao is a small watershed in Mae Sot district, Tak province, northwestern Thailand (Figure 3.1) with mineral-rich, fertile soils and good water resources. Mae Tao Creek flows from the east with a network of canals taking water to the flood plain fields in the west. Eventually, the creek joins the Mei River in the west, at the Thailand-Myanmar (Burma) border. The climate is humid with very high annual rainfall (mean annual temperature 26.4˚C; mean annual humidity is ~75%; mean annual rainfall 1,435 mm; 1977-2008) (Thai Meteorological Department, 2009).

Seasonal rice farming with beans as a secondary crop versus cash crops are the two main cultivation practices in this area. Rice production starts when the rainy season commences, usually in May or June, and ends in December every year. During the dry season (January-April), soy beans, garlic or shallots are grown (Lertlakanawong, 2005).

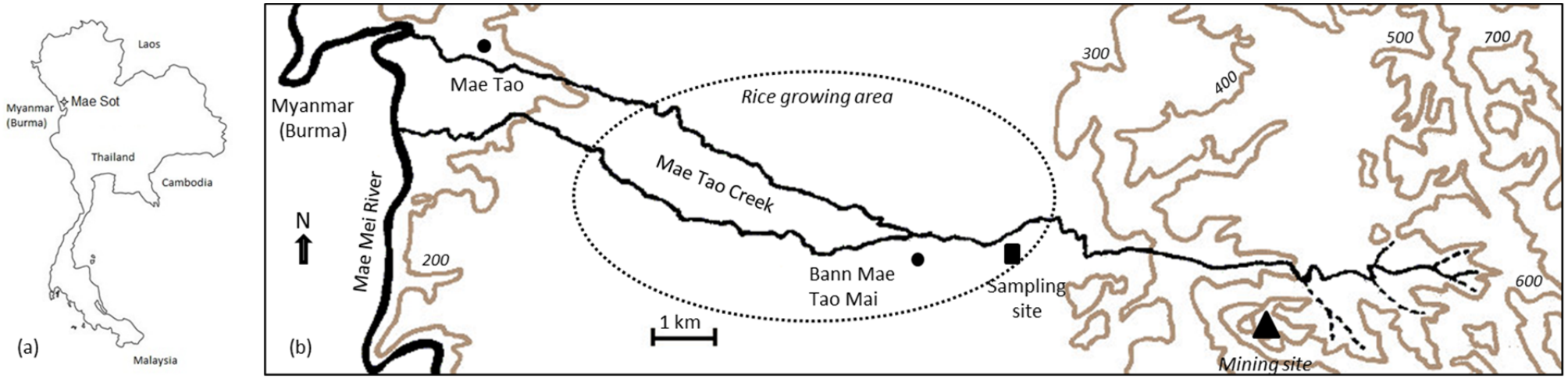


Figure 3.1 Map of (a) Thailand showing Mae Sot district and (b) the Mae Tao watershed within Mae Sot district showing the sampling site (■) and its proximity to the Zn mining site (▲)

Mae Tao watershed (85.7 km^2) is reputedly the best rice growing area in Mae Sot district and has over 35 km^2 of cascade irrigated rice fields with an annual combined rice production of almost 76,000 t (Lertlakanawong, 2005; Simmons *et al*., 2008). A full account of the geological and geographical features of the study site is given in Chapter 2. In brief, the watershed is located on the western side of the Thanon-Thongchai mountain ranges, near the Thailand-Myanmar border. Its geographical features include high mountains with valuable metal-rich areas in the east. The rice-growing areas, located on the floodplains in the western lowlands, are only ~4-15 km from the largest zinc mining area (Padaeng deposit) in Southeast Asia.

The sampling location had to meet the following criteria: (i) be within the Cd-contaminated rice growing zone shown in Figure 3.1(b); (ii) consist of a group of preferably greater than 10 fields; (iii) be supplied with water via canal(s) from the Mae Tao Creek (Figure 3.1(b)); and (iv) have well-maintained check-dams in both the canal(s) and the creek. After consultation with local villagers, a site was selected in the rice-growing areas near Baan Mae Tao Mai village, ~4 km from the mining area. The study site encompassed a well-maintained irrigation check-dam on Mae Tao Creek, which supplies a canal and which in turn feeds a set of 18 paddy fields (Figure 3.2). The distance from the main creek to the paddy fields was about 1 km and the total area of the fields was ~2,400 m^2. The 18 fields comprise a single field set (or group), having one owner who manages the land and leads the water user group for the area.

The dimensions of the Mae Tao Creek at the study site were ~4-8 m wide x ~2 m deep. The canal which takes water from the creek to the paddy fields was smaller and shallower (0.8-2 m wide x 0.3-0.5 m deep). The water enters the field group at the eastern side and spreads across all 18 fields before flowing back to another canal via the lowest lying fields (Figure 3.3).

3.1.2 Sample Collection

In order to understand the behaviour of Cd throughout the rice growing season, samples from the creek, canal and the paddy fields were collected during the: (i) dry period prior to rice planting (July 2010; the rainy season was delayed in 2010); (ii) flood period after which rice growing commenced (August 2010); and (iii) harvest period (November 2010).

Water and sediment samples were collected at two sites along the creek (Cr1-2) and four sites along the canal (Ca1-4) (Figure 3.2) on the dates indicated in Table 3.1. The water samples were collected at a depth of ~30-50 cm below the surface using a pre-cleaned 1 L polyethylene bottle (washed with deionised water then followed by 2% v/v Aristar nitric acid overnight and rewashed with deionised water). Sediment samples were collected at the same sites as water samples by taking grab samples in plastic bags (~1 L) at ~0.5-1.5 m deep which were zipped shut under the water.

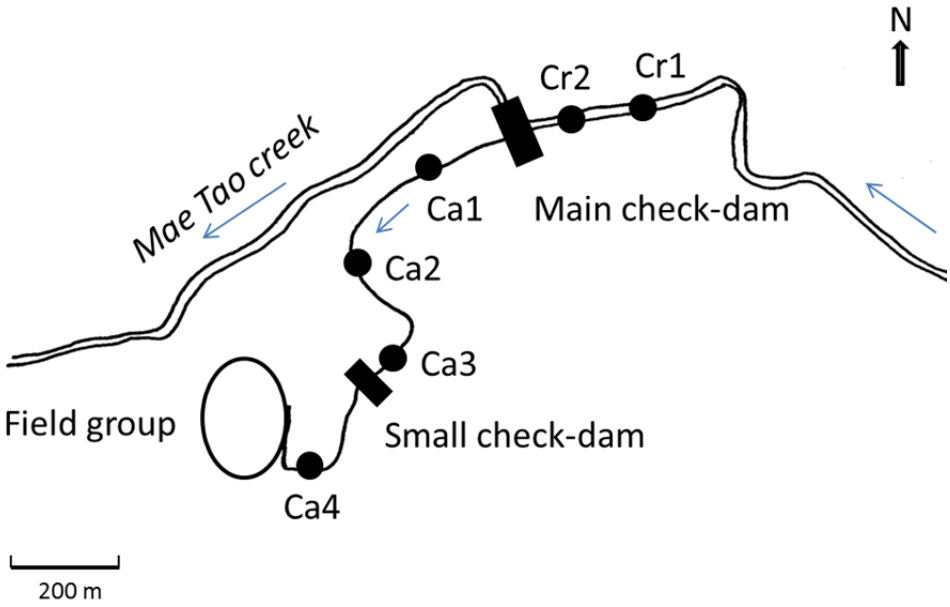


Figure 3.2 Schematic showing the location of water and sediment sampling sites along the Mae Tao Creek and the main canals leading to the field group

Table 3.1 Water and Sediment Samplings in the Dry and Flood periods

Sample		Water		Sediment	
		Dry period 07/10	Flood period 08/10	Dry period 07/10	Flood Period 08/10
Creek	Cr1	✓	✓	✓	✓
	Cr2	✓	✓	✓	*
Canal	Ca1	✓	✓	✓	✓
	Ca2	✓	✓	✓	*
	Ca3	✓	✓	✓	*
	Ca4	✓	✓	✓	✓

*not accessible during flood period

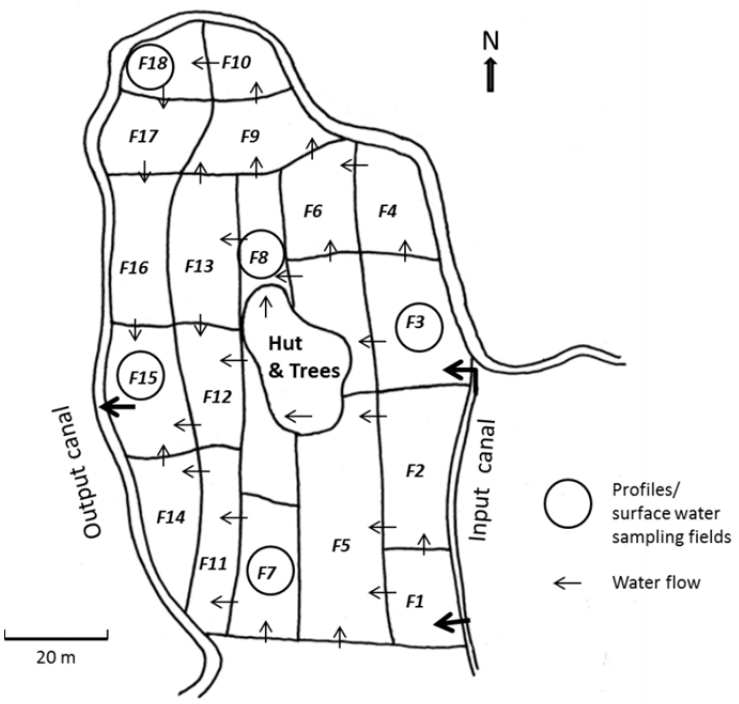


Figure 3.3 Schematic showing the direction of water flow through the group of 18 fields selected for this study. The five fields from which soil profile (0-20, 20-40 and 40-60 cm) and surface water samples were collected are indicated

Topsoil samples (0-20 cm depth) were collected during the dry period in July 2010 using a hand auger at 5-6 sites selected at random in each field (Figure 3.3) and these

were used to generate a separate composite sample (~0.5 kg) for each of the 18 fields (F1-18) (Figure 3.3). For five of the paddy fields (F3, F7, F8, F15 and F18), soils were also collected in July 2010 at depths of 0-20, 20-40 and 40-60 cm to give depth profiles. Again, 5-6 randomly selected sites within each of these 5 fields were sampled at these depths and the material for each depth interval was combined to generate a composite sample for each depth zone for each field. Water samples above the soil surface in the same 5 fields were also collected as a single sample in each field using a pre-cleaned 1 L polyethylene bottle during the flood period in August 2010. Rice plant materials (whole plants including roots and grains) were collected from each of the 18 fields during November 2010. In the same way as mentioned described for soil samples, rice plant samples were collected at 5-6 locations (two rice plants per location) chosen at random within each field. The whole rice plant including the roots was collected and then washed in the field using clean water to remove soil. Then the rice plants were taken to the laboratory where they were washed again using deionised water. After that the whole plant samples were weighed and dried in the oven at 65˚C for about 2 days until they reached a constant weight. The samples were weighed again and prepared for analysis.

A mineral-rich sample was collected in the vicinity of the nearby mining area. In addition, rice fertiliser samples; N-P-K fertilisers with composition 16-20-0 and composition 16-8-8, were obtained from a local market on account of the well-established link between Cd and phosphate fertilisers (McLaughlin *et al*. 1999; Pan *et al*., 2010). After analysis, the Cd concentrations in those fertilisers were found to be lower than the detection limit (<0.01 mg kg^{-1}).

3.1.3 Sample Processing and Analysis

After collection, all samples were brought to the central laboratories of the Faculty of Agriculture, Chiangmai University, Thailand, and processed prior to transporting to Edinburgh, UK, under licence. Suspended particles from water samples were

separated by first using a vacuum filter through 2 μm filter paper (Whatman; cellulose acetate); both the filtered water and the filter papers were stored at 4˚C. Water samples were further filtered through 0.2 μm syringe filters (Whatman; cellulose acetate) and then fractionated by centrifugal ultra-filtration (Vivaspin 20; polyethersulfone membrane) to give a colloidal fraction (3 kDa-0.2 μm) and a truly dissolved fraction (<3 kDa). Soil samples were dried at room temperature and then ground, by using mortar and pestle, to pass through a 2 mm sieve. Sediment samples were stored at 4˚C before transportation to Edinburgh. The sediment samples were wet-sieved into four size-fractions (>2 mm, 53 μm- 2 mm, 20-53 μm, and <20 μm) using deionised water. The >2 mm fraction consisted mainly of decaying vegetation and was discarded. All other fractions were air-dried and ground to homogenise. Rice plant samples were washed with deionised water to remove soil particles and then dried in an oven at 65˚C until constant weight was achieved. Whole plant samples were weighed and then separated into 4 parts (grain, husk, straw and root) which were then weighed individually. All plant samples were finally cut into small pieces and ground into fine powders using a ball mill, with standard cleaning practices (the mill ball was cleaned with 70% v/v ethanol) employed between samples.

3.1.3.1 Geochemical Characterisation of Soils and Water Samples

The general soil/water characteristics were measured in the Chiangmai laboratories. Soil pH was determined in 10 g soil: 20 ml deionised water suspensions using a Sartorius PP- 50 pH meter which had been calibrated using buffer solutions at pH 4 and pH 7. Soil moisture content was measured by oven-drying a sub-sample of every soil sample (5 g soil, 4 h at 105˚C or until constant weight was achieved). All soil and sediment elemental concentrations were calculated using the oven-dry weights. Particle size distribution was determined using a hydrometer (Department of Sustainable Natural Resources, 2010; see Appendix 3.1) Water sample pH and E_h values were measured using a calibrated Combo pH/ORP meter (Hanna). For pH,

calibration was achieved using buffers pH 4.01, 7.01 and 10.01; for E_h, the calibration used was the factory setting.

3.1.3.2 Elemental Concentrations in Water Samples

The concentration of Cd and a suite of other elements (Zn, Pb, Mn, Fe, Cu and Mg) in all water samples, including ultrafiltration samples, were measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Perkin Elmer Optima 5300DV instrument or by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using an Agilent 7500ce instrument, depending on the concentration range.

For ICP-OES, calibration standards (0.01-1 mg L^{-1}) were prepared from 1,000 mg L^{-1} ICP standard solutions (Fisher Scientific). The ICP Multielement Standard Solution VI (CertiPUR®, Merck), which has a certified Cd concentration of 10 mg L^{-1}, was run with every batch of samples. The mean measured Cd concentration was 9.7±0.27 mg L^{-1} (n=9). The wavelengths used and the operating conditions employed during ICP-OES analyses are shown in Tables 3.2 and 3.3, respectively.

Table 3.2 Elemental Wavelengths Used in ICP-OES Analysis

Analysed elements	Wavelength (nm)
Mn	293.305
Fe	239.562
Cu	327.393
Pb	220.353
Zn	206.200
Cd	214.440
Ca	317.933
Mg	279.077
P	178.221
S	181.975

Table 3.3 ICP-OES Conditions Adopted for All Analyses

Instrument	Parameters
Argon flow	
Coolant	15 L min^{-1}
Auxiliary	0.2 L min^{-1}
Nebulizer	0.8 L min^{-1}
Pump flow rate	1.5 L min^{-1}
RF power	1,400 W
Number of replicates	3

For ICP-MS, calibration standards (0.01-10 μg L^{-1}) were prepared from the same stock solutions as for ICP-OES. NIST SRM1643e which has a certified Cd concentration of 6.57±0.07 μg L^{-1} was used for quality control. The mean measured value was 6.63±0.18 μg L^{-1} (n=4). The operating conditions of ICP-MS are shown in Table 3.4.

Table 3.4 ICP-MS Conditions Adopted for All Analyses

Instrument	Parameters
RF power	1,540 W
Reflected power	1 W
Argon gas carrier flow	0.82 L min^{-1}
Argon gas make-up flow	0.21 L min^{-1}
Nebuliser up-take rate	0.2 ml min^{-1} (0.06 rps)
Analyser pressure (vacuum)	3×10^{-6} Pa
IF/BK pressure (vacuum)	8.5×10^{-1} Pa
Rinse speed (rinse port)	0.3 rps
Between sample rinse time, rinse vial	40 sec
Rinse speed (rinse vial)	0.1 rps
Between sample rinse time, rinse port	10 sec
Number of replicates	3

3.1.3.3 Organic Matter and Elemental Concentrations in Solid Samples

Loss on ignition (4 h; 450˚C; muffle furnace) was used as a measure of soil/sediment organic matter content. After dry-ashing 0.25 g of each (in duplicate) soil and sediment sample, including particle size-fractionated samples and suspended particulate matter, microwave-assisted acid digestion using 15.8 M HNO_3 (Aristar) and 22.6 M HF (Aristar) was performed (modified US EPA Method 3052, US EPA, 1996; modification = inclusion of dry-ashing step). Approximately 0.5 g dried and ground rice plant samples (in duplicate), i.e. root, straw, husk and grain, were ashed (4 h; 450˚C; muffle furnace) and were subjected to microwave-assisted acid digestion using 15.8 M HNO_3 and 11.65 M HCl (modified US EPA Method 3051a, US EPA, 2007; modification = inclusion of dry-ashing step). Reagent blanks were included in each microwave digestion run. For each sample type, the extracted

samples and reagent blanks were then transferred to beakers (Teflon for the HF/HNO_3 acid digest). The digestion vessels were rinsed with 2% (v/v) Aristar HNO_3 and the rinsings were added to the sample beakers. Thereafter, the volume was reduced to 1-2 ml on the hotplate. After that, the extracts were transferred to 25 ml polypropylene volumetric flasks, made up to 25 ml with 2% (v/v) Aristar nitric acid before being stored in 30 ml sterilin tubes at 4˚C prior to analysis. All digests of soil, sediment and rice samples were analysed for Cd and other elemental concentrations by ICP-OES (as described above). The values for reagent blanks were subtracted from sample values in each run.

Quality control was achieved via the digestion and analysis of certified reference materials: CRM051-050 (RTC, 2010) for soil and TRM24001 (Yafa *et al.,* 2010) for rice, with measured values closely matching certified values (e.g. 42.5±2.7 mg kg^{-1} (n=7) vs certified value 42.2 mg kg^{-1} and 0.64±0.01 mg kg^{-1} (n=2) vs certified value 0.69 mg kg^{-1} for soil and rice, respectively; Table 3.5).

Table 3.5 Measured and Certified Concentrations of Cd in Soil and Rice Certified Reference Materials

Certified reference material	**Measured Cd concentration (mg kg^{-1})**
For soils: CRM051-50 (certified value = 42.2±0.997 mg kg^{-1})	
#1	38.6
#2	43.4
#3	40.0
#4	44.9
#5	40.7
#6	45.2
#7	44.5
For rice: TRM24001 (certified value = 0.69±0.06 mg kg^{-1})	
#1	0.63
#2	0.65

3.1.3.4 Mineralogical Characterisation of Soil and Sediment Samples

X-ray Powder Diffraction (XRPD, Philips PW1800) was used to quantify the crystalline minerals in selected samples (Zn-rich ore-proxy material; suspended particles and sediment samples (Ca3); high-Cd contaminated soils (F10 and F18)). The XRPD system was used in conjunction with the PC-APD software to collect and analyse the X-ray diffraction data. Quantitative XRPD analysis was performed using Siroquant V2.5 phase analysis software. Selected soil and sediment samples were prepared by grinding by using mortar and pestle into fine powders which were then deposited in a hollow space on a flat plate.

Scanning Electron Microscopy–Energy Dispersive X-Ray Spectroscopy analyses (SEM-EDX, Philips XL30CP with PGT Spirit X-ray analysis and HKL Channel5 Electron Backscatter Diffraction (EBSD) systems) was used to characterise the morphology of the particles on a nanometre (nm) to micrometre (μm) scale. Selected soil (highly contaminated, F10) and suspended particles and sediment (Ca3) samples were dried and carbon-coated before analysis. The topography of the sample was obtained using SEM; the bright spots on the photograph may indicate areas of high concentration of heavy metals and these were selected for analysis of the chemical composition using EDX.

3.1.3.5 Elemental Concentrations in Sequential Extracts from Soil and Sediment Samples

For comparison with soil-plant transfer factors, the solid phase associations of Cd were established via sequential extraction of soil and sediment samples. The European Communities Bureau (BCR) method (Ure *et al.*, 1993) was first selected because the three fractions it targets can be considered to represent potentially

available pools of metal. Low-Cd (F1 and F7), medium-Cd (F4 and F14) and high-Cd contaminated soils (F10 and F18) were extracted in duplicate.

The first step was to extract the exchangeable fraction which is bound to carbonates or otherwise loosely held (Reagent 1: 0.11 M CH_3COOH; pH 2.85). The procedure started by weighing 0.25 g or 0.5 g soil (depending on the soil Cd concentration) into a 50 ml polypropylene centrifuge tube to which 20 ml Reagent 1 was added. The tubes were shaken for 24 hours on a mechanical end-over-end shaker and then centrifuged at 7000 rpm (6793 g) for 15 minutes. The supernatant was decanted from the extracted samples using a syringe, filtered (Millex filter units, 33 ml, 0.2 µm pore size) and then stored at 4ºC for ICP-OES analysis.

The second step was to extract the reducible fraction which is bound to hydrous oxides of Fe and Mn (Reagent 2: 20 ml of $NH_2OH{\cdot}HCl$ 0.1 mol L^{-1}; pH 2). Reagent 2 was added to the solid residue from the first step and then the tubes were shaken for 24 hours. The separation procedure was then performed as described above.

The third step was to extract the oxidisable fraction which is bound to organic matter (Reagent 3.1: 5 ml (two times) of H_2O_2 8.8 mol L^{-1} followed by Reagent 3.2: 30 ml of CH_3COONH_4 1.0 mol L^{-1}; pH 2). 5 ml of Reagent 3.1 was added to the residue from the previous step and left at room temperature for one hour before being placed in hot water (80ºC) for a further one hour. The samples were then allowed to cool before repeating the procedure again. After that 30 ml of Reagent 3.2 was added to sample tubes. All tubes were then shaken for 24 hours before the supernatant was separated following the procedure outlined in the first step.

Residual material from the final step was subjected to microwave-assisted acid digestion using aqua regia (30 ml of 11.65 M HCl : 15.8 M HNO_3, 3:1). Following appropriate dilution using 2% (v/v) Aristar HNO_3, elemental concentrations were determined by ICP-OES as described in section 3.1.3.2 above. For all of the steps,

the reagent blanks were run along with the samples and were subtracted from the sample values.

Initial results from the BCR procedures (see results section) indicated that most Cd was present in the exchangeable fraction (BCR step 1) and so it was decided to subject solid samples to the first two steps of the adapted Tessier sequential extraction scheme (Tessier *et al*., 1979) in order to distinguish truly exchangeable forms (i.e. Cd extractable by a neutral salt, 1 M $MgCl_2$ at pH 7; Tessier step 1) from that bound to carbonates (i,e, Cd extracted by 1 M CH_3COONa at pH 4.5; Tessier step 2).

The first step involves release of exchangeable forms of the element, Here, ~1 g of each soil sample was accurately weighed into a centrifuge tube, 25 ml of 1 M $MgCl_2$ (pH 7) was then added and the sample suspensions were shaken for 2 hours before being centrifuged at 7000 rpm for 15 minutes (6793 x g). After that, the supernatants were filtered using 0.22 µm syringe filters (33 mm Millex® Syringe Filters) before being analysed by ICP-OES (no dilution). The second step involves extraction of carbonate bound forms of the element. In order to avoid re-absorption, the reagent in step 2 was prepared at pH 4.5 rather than pH 5 as in the original method. Here, the residues from the first step were suspended in 25 ml 1 M NaOAc (pH 4.5) and shaken, centrifuged and filtered as described above. The filtered supernatant was diluted 10 times with deionised water prior to analysis by ICP-OES.

As for the BCR procedure, soil samples subjected to Tessier extractions were prepared in duplicate and reagent blanks were also analysed and values subtracted prior to concentration calculations. The typical uncertainty values (error values) for duplicates were <5%.

Six soil samples i.e. F1 and F7 represented the low-Cd contaminated soil (Cd concentration <10 mg kg^{-1}), F4 and F14 represented the medium-Cd contaminated

soil (Cd concentration 10-50 mg kg^{-1}) as well as F10 and F 18 represented the high-Cd soil (Cd concentration >50 mg kg^{-1}). All samples were prepared in duplicate and reagent blanks were also analysed. The typical uncertainty values for duplicates were <5%.

3.1.4 Statistical Analysis

Linear regressions (using IBM SPSS version 22) were carried out in order to analyse the relationship between total Cd, BCR and Tessier-exchangeable versus rice grain Cd for the Cd-contaminated soils. The level of significance was $p<0.05$.

3.2 Results and Discussion

3.2.1 Cd Concentration in Water Samples

In the main, there were only small variations in the water parameters during the study period. The water samples were very clear except during the flood period. The pH was generally slightly alkaline, ranging from ~7.6-8.0. In the flood period, the pH values were slightly lower (~7.6-7.7) in comparison with the other sampling periods (Table 3.6). The water temperature was in the range 23.0-29.3°C during the three periods. The flood period coincides with the highest temperatures (27.7-29.3°C) while the harvest period, occurring in winter had the lowest (22.0-26.0°C) values (Table 3.6). The range of redox potential values was 143-339 mV. The redox potential values in the flood period (235-247 mV) were lower than the dry period (327-357 mV) and the values for the harvest period were the lowest (143-207 mV) (Table 3.6).

Table 3.6 pH Values, Temperature and Redox Potential Values of Creek and Canal Water in Three Periods

Site	Distance (m) from Cr1	Dry Period			Flood Period			Harvest Period		
		pH	Temp (°C)	Redox Potential (mV)	pH	Temp (°C)	Redox Potential (mV)	pH	Temp (°C)	Redox Potential (mV)
Cr1	-	7.80	26.0	339	7.71	28.4	235	7.99	23.0	207
Cr2	112	7.74	26.6	357	N.C.	N.C.	N.C.	7.65	22.7	205
Main check dam	115									
Ca1	276	7.86	26.5	332	7.68	27.7	237	7.95	22.5	164
Ca2	724	7.89	26.6	336	7.70	28.9	240	7.75	24.5	166
Ca3	760	7.69	26.7	327	7.61	28.6	247	7.76	22.0	143
Small check dam	767									
Ca4	1,089	7.84	27.5	327	7.64	29.3	245	7.60	26.0	180

N.C. = not collected

Creek and canal dissolved (<0.2 μm) Cd concentrations were <0.1 μg L^{-1} during the dry (July) and harvest (November) periods, but were ~0.5-6.2 μg L^{-1} during the August flood period (Table 3.7). Suspended particulate Cd was only detected during the flood period, with concentrations of ~4.2-9.7 μg L^{-1} (Table 3.9). Figure 3.4 shows that flood period total Cd concentration (dissolved+suspended) decreased with increasing distance along the irrigation channel from the main creek and that suspended particulate Cd comprised >80% of total Cd in the canal water samples (Ca1 and Ca4). Although total Cd decreased with distance, concentrations were still considerably greater than would be expected for unpolluted water (<1 μg L^{-1}: Friberg *et al*., 1986) even at ~1.1 km from the main creek (i.e. 4.2 μg L^{-1}, with >89% in the particulate phase; Figure 3.4). With the exception of the flood period creek water sample Cr1, dissolved (<0.2 μm) Cd in creek and canal water was typically <1 μg L^{-1} with 91.9±1.3% (5 samples) present in truly dissolved forms (<3 kDa).

Flood period surface water samples from the fields (F3, F7, F8, F15 and F18) had Cd concentrations and distributions similar to those of Ca4. Across the five fields, dissolved (<0.2 μm) Cd concentration averaged 0.18±0.04 μg L^{-1} (range 0.09-0.32

µg L^{-1}; Table 3.8). Cd concentrations in suspended particulates in fields F7 and F18 were 3.30 and 2.49 µg L^{-1}, comprising 96.7 and 94.7%, respectively, of total Cd in the water (Table 3.9 and Figure 3.4). Despite recent improvements in mining practices, e.g. the construction of additional emergency ponds to contain storm water in the mining area and the construction of dykes around its perimeter to prevent soil erosion (Padaeng Industry Public Company Limited, 2007), the water entering the fields is still transferring Cd, mainly in association with suspended particulate matter, to the paddy fields.

Table 3.7 Cd Concentration in the Dissolved (<0.2 µm) Phase of Water Samples Collected during the Three Sampling Periods

Site	Distance (m) from Cr1	Cd concentration (µg L^{-1})		
		Dry period (in July)	Flood period (in August)	Harvest period (in November)
Cr1	-	0.02	6.24	0.097
Cr2	112	0.01	N.C.	0.04
Main check dam	115			
Ca1	276	0.01	1.13	0.03
Ca2	724	0.01	0.84	0.17
Ca3	760	0.005	0.68	0.02
Small check dam	767			
Ca4	1,089	0.01	0.50	0.01

N.C. = not collected

Table 3.8 Cd Concentration in the Dissolved (<0.2 µm) Phase Surface Water in Fields in Flood Period

Surface water in fields	Cd concentration (µg L^{-1})
F3	0.32
F7	0.19
F8	0.20
F15	0.13
F18	0.09

Table 3.9 Cd Concentration of Dissolved and Suspended Particles in Water Creek and Canal in Flood Period (mean ± standard error; n=2)

Site	Distance (m) from Cr1	Cd concentration (µg L^{-1})		
		Dissolved (< 0.2 µm)	Suspended particles	Dissolved+ Suspended
Cr1	-	6.24±0.036	7.78±0.23	14.0
Cr2	112	N.C.	-	-
Main check dam	115			
Ca1	276	1.13±0.002	9.75±0.15	10.9
Ca2	724	0.84±0.02	N.D.	-
Ca3	760	0.68±0.01	N.D.	-
Small check dam	767			
Ca4	1,089	0.50±0.005	4.18±0.09	4.68
F7	1500	0.17±0.004	3.30±0.23	3.49
F18	1550	0.09±0.004	2.49±0.10	2.58

N.C. = not collected; N.D. = not determined

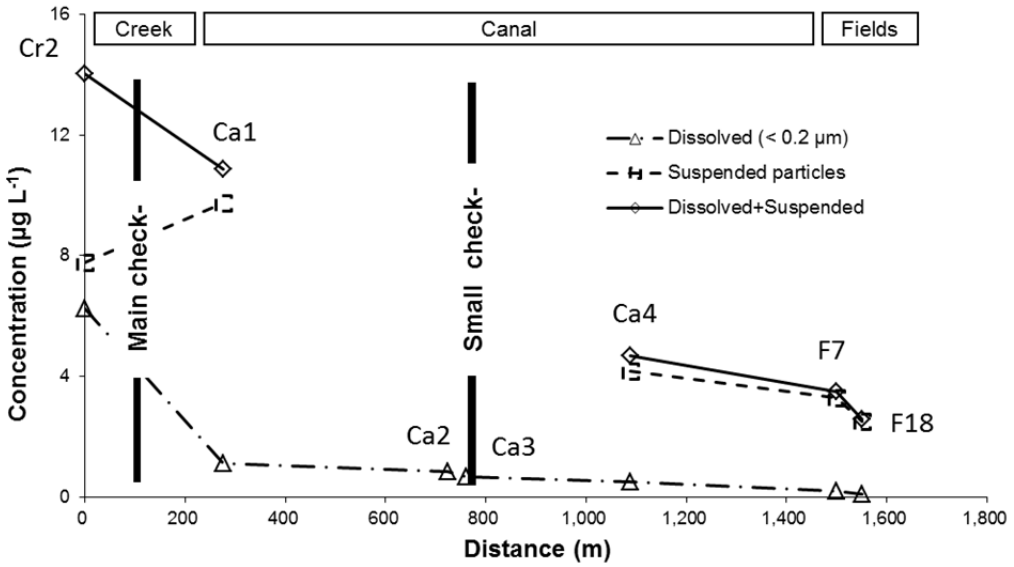


Figure 3.4 Cd concentration (µg L^{-1}) in the creek, canal and soil surface water during the flood period. The x-axis indicates distance from creek sampling point Cr1, which is upstream of the main check-dam. Suspended particles at three of creek and canal sites (Cr1, Ca1 and Ca4) were analysed during the flood period.

3.2.2 Soil Properties, Element Concentrations and Mineralogy in Sediment, Soil, Particulate & Mineral Ore Samples

3.2.2.1 Soil Properties

The topsoil (0-20 cm) from the 18 fields had a near-neutral to slightly alkaline pH (6.8-7.7), organic matter content 0.65-4.75%, (Table 3.10). Six selected soils had a mostly loamy texture with 16-27% clay (Table 3.11). The soil moisture content in the dry and harvest period ranged from 8.9-21.2% and 13.6-28.0%, respectively (Table 3.10).

Table 3.10 pH, Organic Matter and Moisture in Fields (topsoil 0-20 cm; mean of duplicate ± standard error)

Soils	pH (Dry period)	Organic matter (%) (Dry period)	Moisture (%)	
			Dry period	Harvest period
F1	7.68±0.02	-	10.3±0.88	18.3±0.46
F2	7.67±0.01	3.74±0.47	10.4±0.72	14.0±0.37
F3	7.55±0.00	-	11.2±0.39	17.2±0.76
F4	7.52±0.07	4.15±0.35	11.46±0.57	18.8±0.34
F5	7.50±0.04	3.95±0.15	8.86±0.38	16.6±0.04
F6	7.41±0.05	3.20±0.50	9.87±0.11	13.6±0.97
F7	7.27±0.03	4.55±0.25	10.4*	16.3±0.16
F8	7.25±0.05	3.00±0.30	10.1±1.01	13.6±0.03
F9	7.63±0.03	3.10±0.20	15.1±0.37	18.4±0.36
F10	7.70±0.03	4.75±0.05	15.2±0.53	19.8±0.50
F11	7.71±0.00	4.50±0.30	13.2±0.27	18.1±0.23
F12	6.84±0.11	4.25±0.85	11.0±0.33	15.3±0.11
F13	6.89±0.04	5.55±0.15	10.6±0.22	14.5±0.39
F14	7.60±0.00	1.25±0.15	15.6±0.07	22.5±0.25
F15	7.47±0.02	4.10±0.40	21.2±0.47	28.0±0.77
F16	6.91±0.00	0.65±0.25	16.3±0.16	21.0±0.49
F17	7.45±0.02	4.70±0.10	15.3±0.23	21.2±0.32
F18	7.70±0.02	3.60±0.90	13.0±0.22	18.7±0.20

* n=1

Soil pH decreased from 7.68 to 7.25 going from F1, where the canal water enters the field group, to F8. In most of the remaining fields except F12, F13 and F16, the soil pH was ~7.5-7.8. Fields F12, F13 and F16 are located in the centre of the field group and had the lowest pH values (6.84-6.89) (Figure 3.5a). The organic matter content of soils in most fields was in the range 3.0-4.8% whilst much lower values were measured for soils from F14 and F16 (1.25% and 0.65%, respectively). These fields were in the lowest zone in the field group. As expected, the soil moisture content in

the harvest period was higher than in the dry period. However, there was a clear relationship between the values obtained during the two periods. In the harvest period, the moisture content was highest (>20%) in the lowest-lying fields, i.e. F14-F17, which were also close to the water outlet (Figure 3.5c and Figure 3.3).

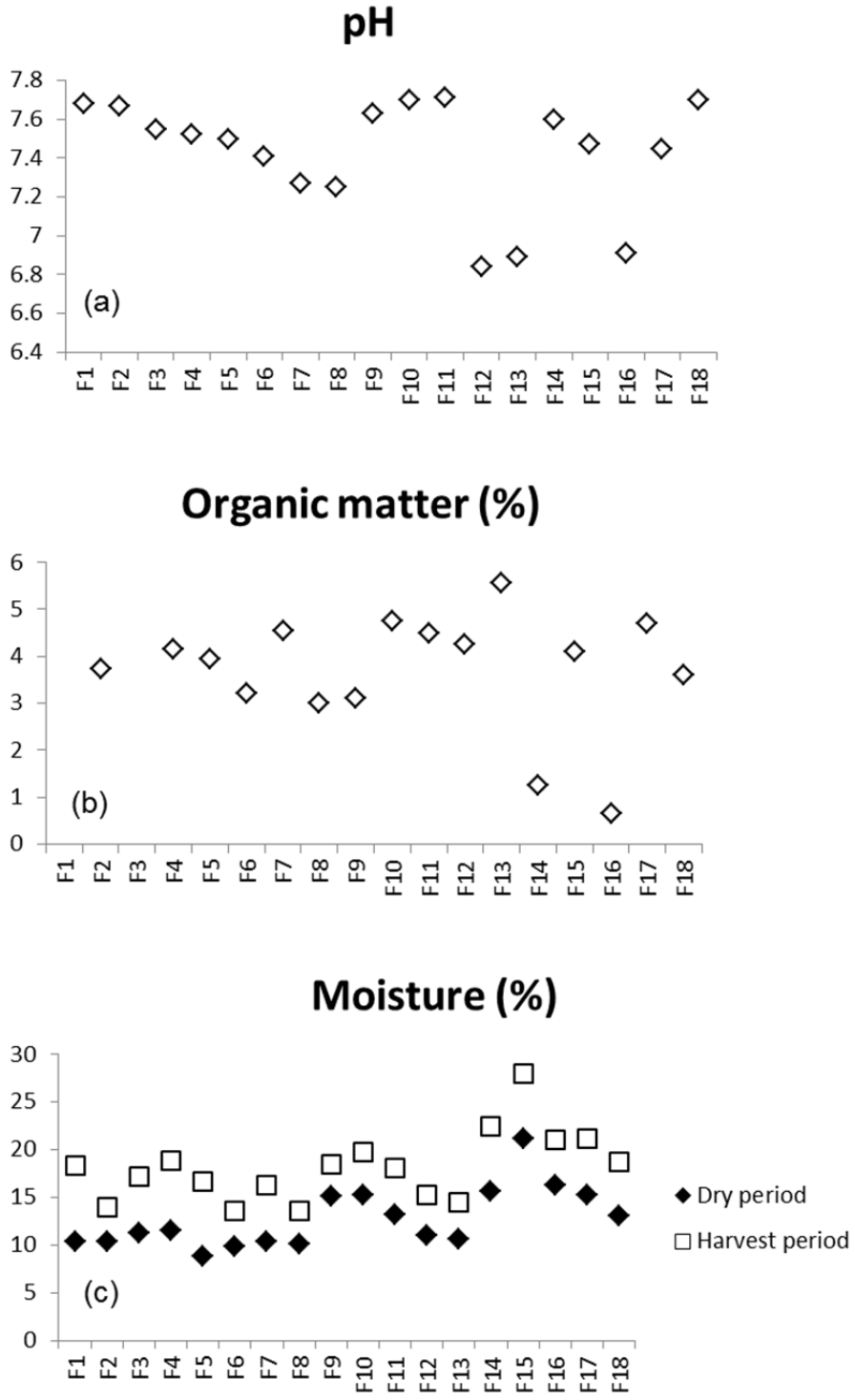


Figure 3.5 Mean topsoil (0-20 cm) (a) soil pH and (b) organic matter content and (c) moisture content in dry period

With regard to the soil texture, the high-Cd fields (F10 and F18) were classified as sandy loams since they had a lower clay content (~16% vs 22-26% for the other fields) but a greater sand content than the others (56-60% vs 40-52% for the other fields). The previously lowest-lying fields including, F10 and F18, had been artificially raised using the top soils from the highest-lying fields (F1, F2 and F3) close to the input canal in order to adjust land slope for more effective dispersal of irrigation water (Boonruang Tawong, pers. comm., 2010). Based on the work by Simmons *et al*. (2005), the soil that has been transferred is likely to have been the most heavily contaminated with Cd from the mining activities in the mountains and it appears that it is also coarser-textured than in the other fields.

Table 3.11 Soils Texture of Low, Medium and High-Cd Contaminated Soils

Cd-contaminated Soils		% Clay	% Silt	% Sand	Texture
Low-Cd	F1	26	34	40	loam
	F7	27	27	46	sandy clay loam
Medium-Cd	F4	22	26	52	sandy clay loam
	F14	24	30	46	loam
High-Cd	F10	16	24	60	sandy loam
	F18	16	28	56	sandy loam

3.2.2.2 Elemental Concentrations in Sediments and Sediment Size Fractions

Total Cd in the sediments (7.14-26.7 mg kg^{-1}) were calculated from the sum of Cd concentration, taking account of the mass present in each of the three size fractions (53 µm - 2 mm, 20 - 53 µm and <20 µm) (see Appendix 3.3). The results revealed a similar trend to that obtained for the suspended particulates in the creek and canal waters. Concentrations were lower upstream (~16 mg kg^{-1}) of the main check-dam

than downstream (~27 mg kg^{-1}), but decreased again towards the small check-dam (~20 mg kg^{-1}) and even further to ~8 mg kg^{-1} at Ca4, some 1.1 km from the main creek (Figure 3.6). Thus, considerable amounts of Cd-contaminated sediment settle out from the incoming waters and accumulate between the main and small check-dams. Elevated Cd concentrations were measured in all sediment size fractions (Table 3.12); the lowest concentrations of Cd were obtained for the largest size fraction (53 μm – 2 mm; Table 3.12) with higher concentrations were observed in the smaller size fractions (Table 3.12) and, because a greater proportion of the sediment was in these smaller fractions, they constituted the bulk of the total sediment Cd load. These finer sediment fractions are likely to be the most readily re-suspended and so it is likely that these could become a source of Cd to the paddy fields during channel clearing activities performed by the villagers annually.

Table 3.12 Cd Concentrations in Size Fractions from Creek (2 samples) and Canal (4 samples) Samples Collected During the Dry Period (July 2010)

Sediment size fraction	% mass	Cd concentration (mg kg^{-1})	% of total sediment Cd (by weight)
53 μm - 2 mm	15.0 – 39.2	4.1 - 23.2	17.5 - 31.5
20 - 53 μm	47.5 – 72.0	12.6 - 36.3	24.9 - 55.2
<20 μm	11.1 – 20.8	9.5 - 34.1	23.8 - 52.3

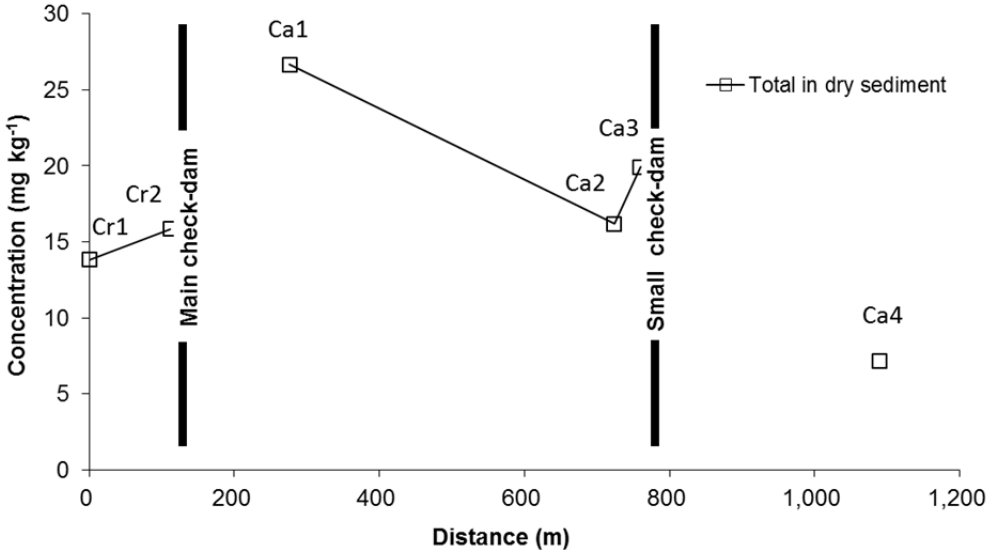


Figure 3.6 Cd concentration (mg kg^{-1} dry mass) in the creek (Cr) and canal (Ca) sediments collected during the dry period. The x-axis indicates distance from creek sampling point Cr1, which is upstream of the main check-dam

3.2.2.3 Cd and Other Elemental Concentrations in Soils from the 18 Paddy Fields

Within the group of 18 fields, there was a wide range of soil Cd concentrations (2.5-87.6 mg kg^{-1}) (Figure 3.7 (a) and Appendix 3.4). Contamination was evident to a depth of 40 cm (Figure 3.7 (b)). The maximum concentration is lower and the range of values considerably narrower than the 0.5-284 mg kg^{-1} reported for soils from 524 fields elsewhere in the region, the Baan Pha Deh, Mae Tao watershed (Simmons *et al*., 2005). However, the Baan Pha Deh site is closer to the mining area than the location of the present study (Baan Mae Tao Mai). Nevertheless, all of the fields in our study site had Cd concentrations greater than the background values for Thai soils (0.03 mg kg^{-1}; Prakongkep *et al*., 2008). Moreover, the concentrations were also

higher than the predicted no effect concentrations (PNECs) for soil Cd determined by Smolders *et al.* (2009). Figure 3.7 (a) also shows that the lowest lying fields have the highest Cd concentrations. Considering the site history (obtained from local records) and method of irrigation, this accumulation pattern may be attributed to (i) increased settling of suspended particles in the lower areas and (ii) landscaping operations for improved irrigation that saw topsoil from elevated lying fields (closer to the source of contamination) being excavated and transferred to lower lying fields (Boonruang Tawong, pers. comm., 2010).

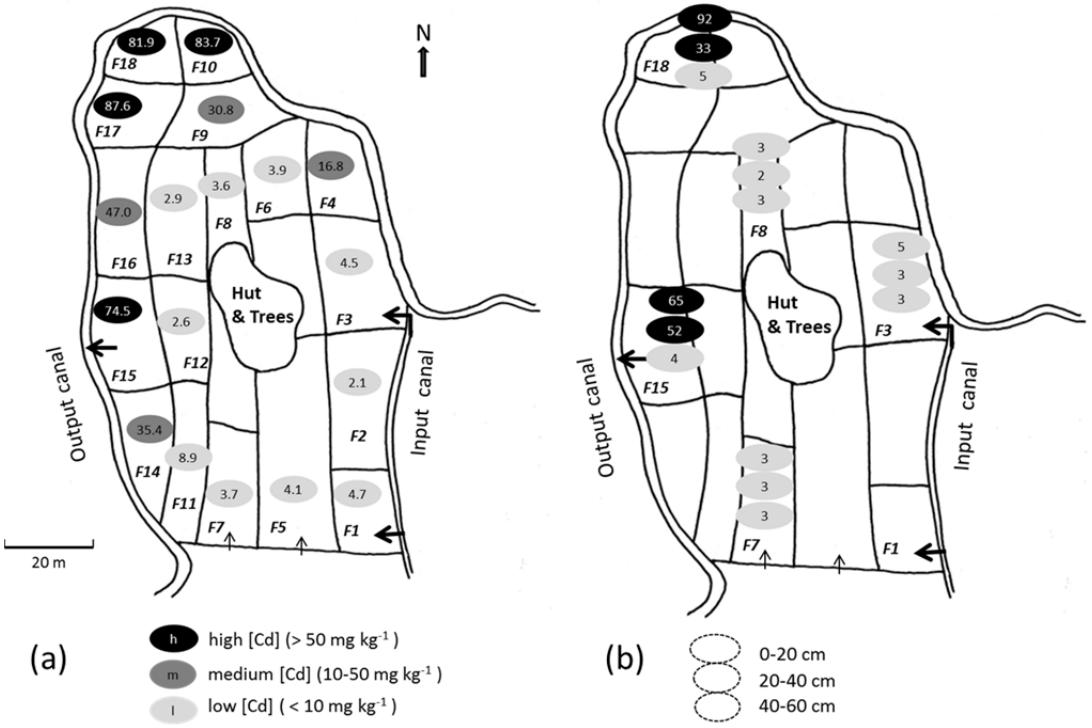


Figure 3.7 Cd concentration (mg kg^{-1}) in (a) topsoil (0-20 cm) and (b) soils from different depths from 5 fields (F3, F7, F8, F15 and F18)

Of the other elements analysed, Fe, Mn, Mg and P concentrations (13.3-21.3 g kg^{-1}, 288-971, 47-3,944 and 191-401 mg kg^{-1}, respectively) (Appendix 3.4) were found to be similar to those generally found in Thai paddy soils (Prakongkep *et al.*, 2008). Concentrations of Cu and Pb were slightly higher (13.1-49.2 and 26.0-141.9 mg kg^{-1},

respectively) and those of Zn, Ca and S were very much higher (125-2,590, 13,900-29,000 and 112-568 mg kg^{-1}, respectively) than commonly observed in Thai paddy soils (Appendix 3.4). Importantly, the fields with the highest Cd concentrations also had elevated concentrations of Zn, Mg, S, Ca, and Pb (Figure 3.8).

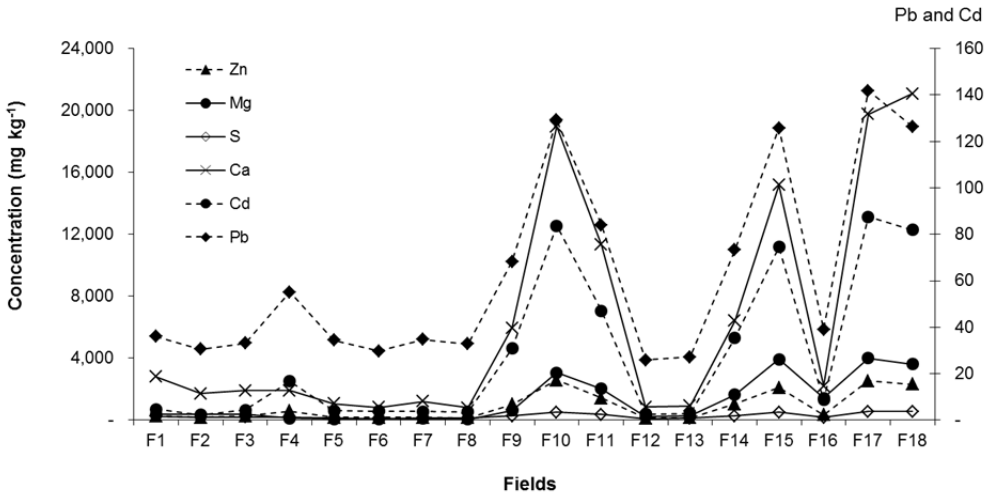


Figure 3.8 Mean concentrations of Zn, Mg, S, Ca, Pb and Cd in topsoil from each of the 18 paddy fields (n=2)

3.2.2.4 Cd and Other Elemental Concentrations in Proxy-ore Material from the Mining Area

In addition to the high concentrations of Zn (~12% w/w) and Cd (~0.3% w/w), the solid phase sample from the vicinity of the mining areas contained high concentrations of Ca, Mg and S (3.4%, 0.86% and 0.09% w/w, respectively) (Table 3.13). As discussed above, the highest Zn, Ca, Mg and S concentrations were found in the fields with the highest Cd contents, demonstrating a clear link between the source material and the contamination in the paddy field soils. The highly contaminated fields also had elevated pH values (Figure 3.5a), most likely as a

consequence of the high carbonate content of the mining-derived contamination (cf smithsonite, one of the main minerals, is a zinc carbonate).

Table 3.13 Elemental Concentrations in Proxy-ore Material from the Mining Area (mean ± standard error; n=2)

Elements	Units	Concentration
Cu	mg kg^{-1}	30.2±0.2
Fe	% w/w	2.57±0.09
Mn	% w/w	0.23±0.001
Pb	mg kg^{-1}	429±10.0
Zn	% w/w	11.7±0.02
Cd	% w/w	0.3±0.01
Ca	% w/w	3.45±0.02
Mg	% w/w	0.86±0.11
P	mg kg^{-1}	311±9.2
S	mg kg^{-1}	937±20.1

3.2.2.5 Mineralogical Characterisation of the Source Material, Creek Suspended Sediments, Canal Sediments and Paddy Field Soils

The source material was further characterised using XRPD analysis. This showed that the main Zn-containing ores were smithsonite ($ZnCO_3$ ~12.5% w/w) and hemimorphite ($Zn_4Si_2O_7(OH)_2$ ~3.1% w/w) (Table 3.14), consistent with secondary deposits in colloform bands derived from weathering of sulphide materials, i.e. galena (PbS), pyrite (FeS_2), and sphalerite (ZnS) (Paijitprapapon *et al.*, 2006). Other major minerals present in the source sample were calcite ($CaCO_3$ ~0.3% w/w), dolomite ($CaMgCO_3$ ~16% w/w) and the phyllosilicates, clinochlore (14.1% w/w), kaolinite (2.3% w/w) and chlorite (4.5% w/w) (Table 3.14).

Table 3.14 Percentage Mineral Composition of Zn-rich Rock Material (proxy-ore) from the Mining Area, Suspended Particles from the Mae Tao Creek, Sediment Samples from the Canal (Ca3) and Soil Samples from Selected Fields (F10 and F18)

Mineral (%)	Zn-rich material	Suspended particles in creek water	Sediment sample Ca3		High-Cd field F10		High-Cd field F18	
		Ca3	(20-53 µm)	(<20 µm)	bulk	(<20 µm)	bulk	(<20 µm)
Quartz	46.6	59.2	90.7	67.1	85.8	39.2	84.6	37.9
Calcite	0.30		5.0	6.0	5.6	14.2	5.7	12.5
Dolomite	16.6		4.3	2.5	3.1	4.9	2.5	3.8
Hemimorphite	3.08							
Smithsonite	12.5							
Clinochlore	14.1							
Kaolinite	2.3	2.69						
Chlorite IIb	4.5	2.19		4.6	1.1		0.9	
Albite		0.09						
Illite		31.5				41.7		45.9
"Microcline maximum"		1.88						
Orthoclase		2.06						
Muscovite		0.33		19.7	4.4		6.3	

Smithsonite and hemimorphite were not detected in any of the creek suspended particulate matter, canal sediments or paddy soil samples. However, some of the other minerals found in the source material were also present at high concentration in the canal sediments. Only the smaller sediment size fractions were analysed as these made up the majority of the sediment. Dolomite and calcite were found at 4.3% and 5.0%, respectively, in the 53-20 µm fraction and at 2.5% and 6%, respectively, in the <20 µm size-fraction (Table 3.14). The mica group, i.e. chlorite and muscovite, were also found in high concentration (4.6% and 19.7%) in the <20 µm size-fraction.

Although there was an absence of detectable calcite and dolomite, suspended particles (>0.2 μm) from the creek water contained quartz, kaolinite, chlorite, albite, illite (~32%), microcline maximum, orthoclase, and muscovite (Table 3.14).

XRPD analysis of soils from the paddy fields that had a high level of Cd contamination (F10 and F18) showed that calcite and dolomite were present in the <20 μm size-fraction at concentrations of 12.5-14.2% w/w and 3.8-4.9% w/w, respectively. Since there is a strong positive correlation between Cd, Zn and Ca concentrations across the entire field group, the higher concentrations of calcite in F10 and F18 soils in comparison with those in the creek suspended particulate matter and canal sediments is most likely due to accumulation of contaminants over time. However, as for all the fields, a portion of the Ca present will be attributable to liming of the soils. Minerals from the phyllosilicate group such as chlorite and muscovite were present only at ~1% and 4-6%, respectively, in bulk samples of these highest Cd soils (Table 3.14). Illite, a 2:1 clay which is structurally similar to muscovite and which was identified in the creek and canal samples, was also found at high concentration (~42-46%) in the <20 μm fraction of the highly contaminated paddy field soils (F10 and F18).

To establish the importance of these minerals with respect to Cd associations, SEM-EDX analysis of selected sediment (Ca3; 20-53 μm) and soil (F10) samples was carried out. Two forms of Cd were identified within both sample types; association with: (i) clay-rich particles which contained Cd together with Al, Si and O; and (ii) mixed aggregates containing both $CaCO_3$ and clay particles which contained Cd together with Ca, Al, Si, O and C. These forms (Cd-Clay and Cd–$CaCO_3$) were found both in the sediments (20-53 μm size fraction) behind the small check dam and in the high-Cd contaminated soils from F10 (Figure 3.9). Although many studies have implicated sulfide phases as a primitively Cd solid phase in soils (e.g. Chaney *et al.*, 1996; Simmons and Pongsakul, 2003), Khaokaew *et al.* (2011) reported that Cd carbonates, specifically Cd-$CaCO_3$ and $CdCO_3$, became the dominant solid phase after Cd-rich soils were flooded for 7 days. Additionally, kaolinite was identified as a

major Cd-bearing phase, accounting for 22% of total Cd in their soils. In this study, it is more likely that illite, which is present in both the soils and suspended particulate matter, is the clay with which Cd is associated.

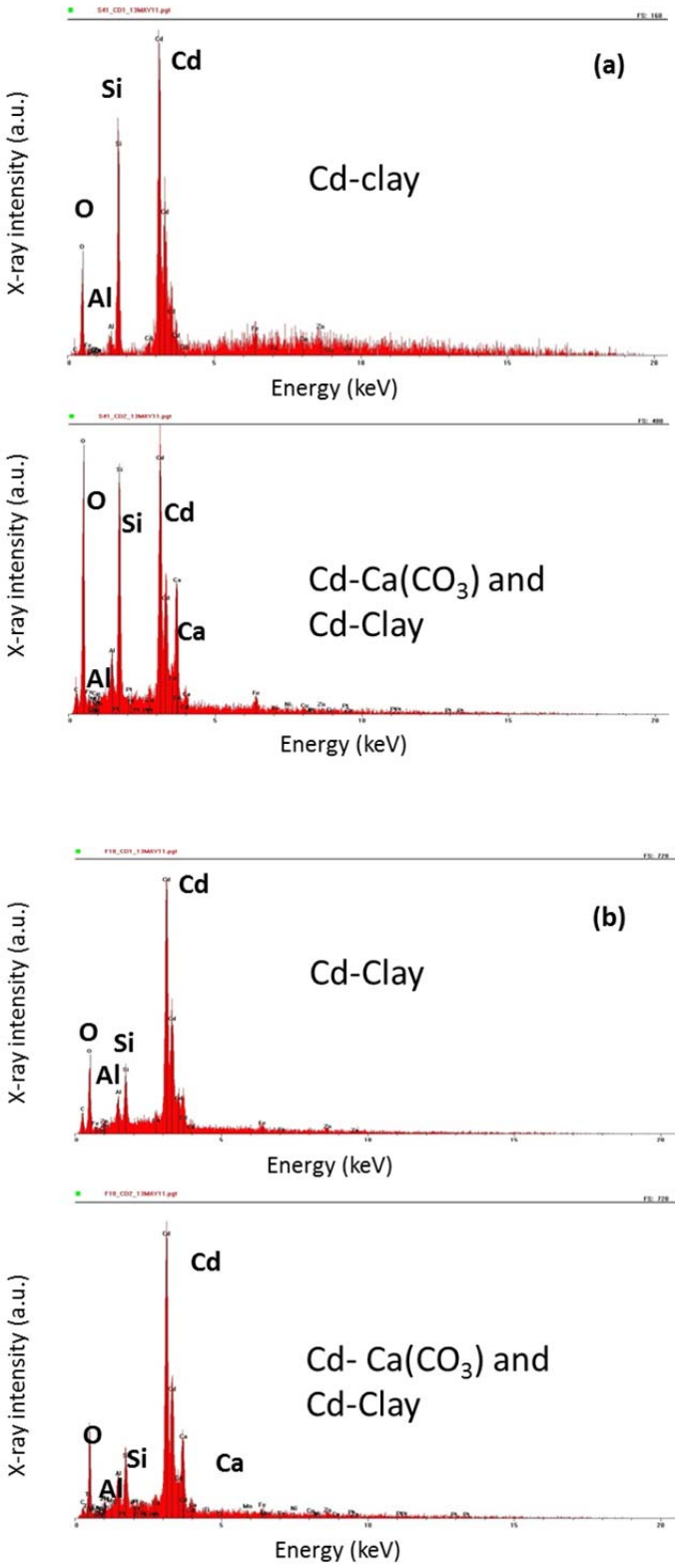


Figure 3.9 SEM-EDX spectra for (a) topsoil (F10) and (b) sediment (Ca3: 20-53 µm) samples

3.2.3 Associations of Cadmium in Paddy Soils and Canal Sediments

The concentrations of Cd in each of the exchangeable, reducible, oxidisable and residual fractions along with the total concentrations obtained by HF digestion of the samples, are given in Table 3.15. Exchangeable Cd, as defined by the first step of the European Communities Bureau of Reference (BCR) scheme (Ure *et al*., 1993), accounted for the majority of Cd in the paddy soil samples tested (~44-78%), followed by reducible or iron/manganese oxide fraction (~15-27%) (Figure 3.10). A good mass balance was observed when the sum of BCR fractions and the total Cd concentration determined by mineral acid digestions were compared, with the sum of fractions mostly 89-110% (averaging 89.2%) of total Cd (Table 3.15; Figure 3.10). Predominance of Cd in the BCR-defined exchangeable fraction indicates a high labile and bioavailable proportion in the soils.

Table 3.15 Cadmium Concentrations in Fractions Determined by the BCR Sequential Extraction Scheme Applied to Soils from F1, F4, F7, F10, F14 and F18 (mean ± standard error; n=2)

Contaminated Soils		Cd Concentration (mg kg^{-1})				Total Cd (HF)	Mass balance (%)
		Step 1: Exchangeable	Step 2: Reducible	Step 3: Oxidisable	Residual		
Low-Cd	F1	1.97±0.05	1.95±0.03	0.40±0.40	ND	4.67 ±0.08	92.5
	F7	0.64±0.01	0.80±0.01	ND	ND	3.70 ±0.06	38.9
Medium-Cd	F4	10.6±0.5	4.35±0.12	ND	ND	16.8±0.47	89.0
	F14	22.1±0.23	9.66±0.14	1.58±0.01	ND	35.4±4.34	94.2
High-Cd	F10	72.0±2.05	17.3±0.07	3.16±0.000	ND	83.7±0.44	110.5
	F18	68.2±0.94	18.8±0.51	3.18±0.005	ND	81.9±4.74	110.1

ND = not detected

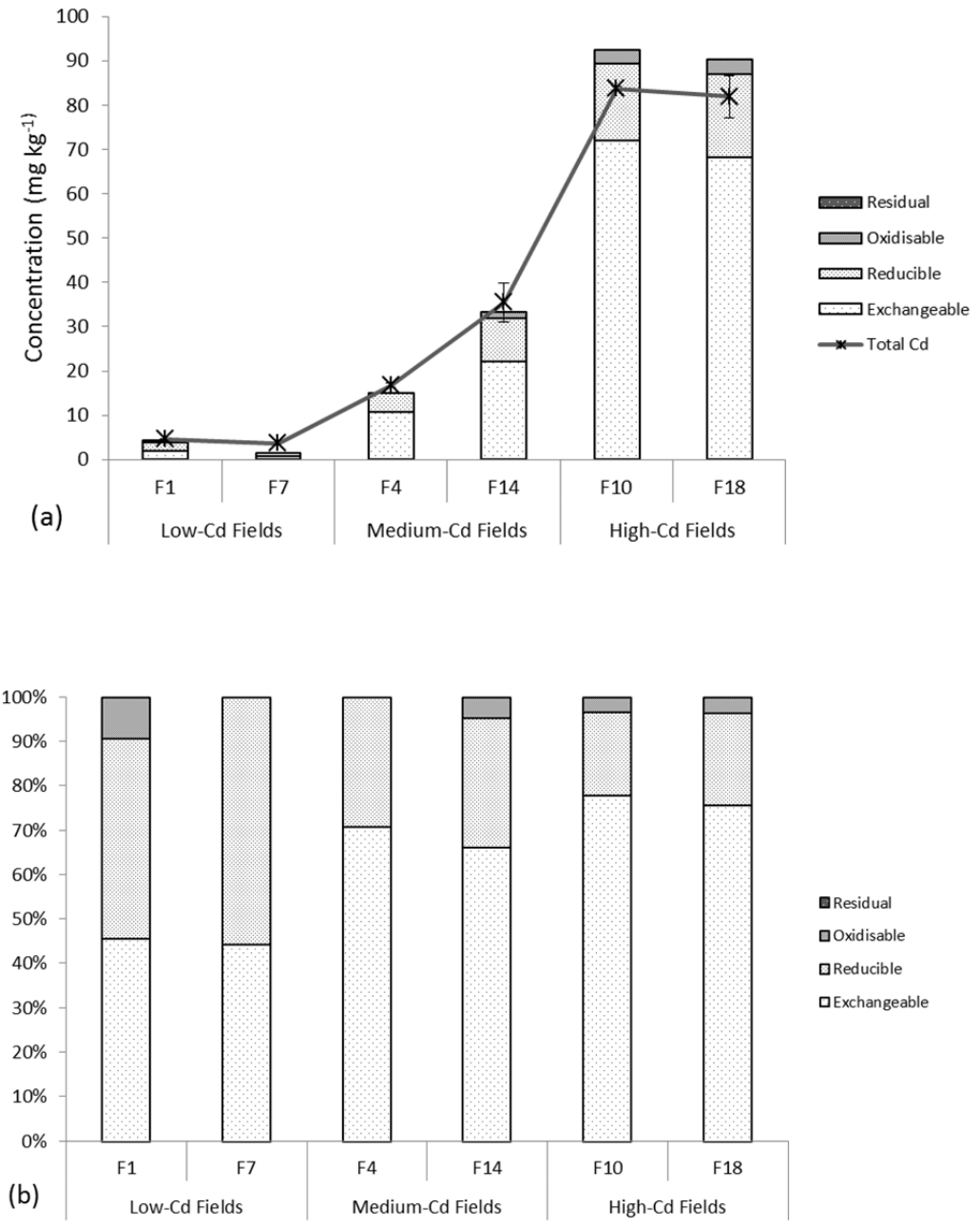


Figure 3.10 Cd associations in soils determined by the BCR sequential extraction scheme: (a) total (HF/HNO_3) Cd concentration (line) and Cd concentrations in BCR fractions and aqua regia-digested residual phases (stacked bars) (b) percentage distribution amongst BCR fractions and aqua regia-digested residual phase

However, as discussed in Section 3.1.3.5, the exchangeable fraction as defined by the first step of the BCR scheme includes both water- and weak acid-soluble forms. The latter includes Cd which is associated with carbonates, which the mineralogical analysis indicated are particularly important in these soils. For this reason the first two steps of the Tessier sequential scheme (Tessier *et al.*, 1979) were used to distinguish the easily exchangeable forms of Cd from that bound to carbonates in both paddy soil and canal sediment samples. The results of the Tessier extractions (Table 3.16) indicated that 28.5 and 45.2% of total Cd was in the Tessier-exchangeable fraction of the two medium-Cd contaminated soils tested, while lower percentages were observed in the high-Cd contaminated soils (24.3 and 21.7%). The carbonate-bound forms accounted for a large proportion in the medium-Cd contaminated soils (45.4 and 46.1%) and an even greater proportion of total Cd in the high-Cd contaminated soils (72.4 and 70.9%; Table 3.15). The total mass of Cd in the exchangeable fraction was within the same range for the medium and high-Cd soils, whereas the mass in the carbonate bound forms were much higher in the high-Cd contaminated soils.

Table 3.16 Cd Associations in Sediments and Paddy Soils Determined by Tessier Sequential Scheme (mean ± standard error; n=2)

Sources		**Exchangeable (T1)**		**Bound to carbonate (T2)**	
		mg kg^{-1}	**(%)****	**mg kg^{-1}**	**(%)****
Sediments (Ca3)	20-53 μm*	1.09	3.00	16.0	44.2
	<20 μm	11.8±0.37	35.9±2.01	5.52±0.14	16.8±0.29
Medium-Cd contaminated soils	F4	7.58±0.18	45.2±0.90	7.62±0.52	45.4±7.79
	F14	10.1±0.06	28.5±0.05	16.4±0.38	46.1±2.00
High-Cd contaminated soils	F10	20.4±0.27	24.3±0.43	60.6±0.61	72.4±2.19
	F18	17.8±0.24	21.7±0.34	58.1±0.33	70.9±0.67

**(n=1)* ** % of total Cd

In the canal sediment (Ca3), the 20-53 μm size-fraction of the sediment behind the small check-dam had 3% Cd in the Tessier exchangeable form and 44.2% bound to carbonates (as defined by Tessier; Table 3.16). For the finer fraction (<20 μm), the

percentage of exchangeable form was 35.9% while that bound to carbonate was 16.8% (Table 3.16). The sediment Cd partitioning results reflect, to some extent, the mineralogy of the size fractions, as the 20-53 μm size-fraction had a higher percentage of carbonates than the finer fraction (Table 3.16).

The mineralogy and sequential extraction results however were in contrast to those of Akkajit and Tongcumpoo (2010), who reported the highest proportions of Cd in the paddy soils they examined associated with Fe and Mn oxides fraction (the BCR2 fraction).

3.2.4 Cadmium Assimilation and Distribution in Plants

Cadmium was readily assimilated by plants and accumulated to differing degrees in different plant tissues (Table 3.17). Cadmium concentrations in roots were highest (~5.4-132 mg kg^{-1}), directly reflecting the level of Cd in the paddy soils. Lower concentrations were observed in other plant parts (~0.02-4.3 mg kg^{-1}).

Grain Cd as a percentage of root Cd concentrations ranged from ~1.3-~3.6% (Table 3.17). This mirrors results for rice plants grown in laboratory experiments (Fujimaki *et al.*, 2010) and in the field for other plant species such as soybean (Oliveira *et al.*, 1994) and sugarcane (Xia *et al.*, 2009), in that only a small proportion of the root Cd is translocated to other plant parts. Although a low percentage of total rice plant Cd is transferred to grains, the grain Cd concentrations in the medium- and high-Cd contaminated fields were significantly above the recommended safe limit of 0.4 mg kg^{-1} (FAO/WHO, 2006). Even the Cd concentrations in rice grains in low-Cd contaminated fields ranged from 0.07-0.6 mg kg^{-1}.

Indeed the Cd levels in rice grains followed the Cd levels in soils (Figure 3.11), with the greatest concentrations (2.8 and 4.0 mg kg^{-1}; Appendix 3.5) occurring in grains

from high-Cd contaminated fields. The Cd concentration in rice grains in low-Cd and medium-Cd contaminated fields ranged from 0.07 to 0.9 mg kg^{-1} (Figure 3.11).

Transfer coefficients (concentration in grains / concentration in soils) were in the range 0.03-0.05 for high-Cd soils and 0.01-0.03 for low-Cd soils and, in the main, Cd in rice grains followed that in the soils (Table 3.17), with the greatest concentrations (2.8 and 4.0 mg kg^{-1}) occurring in grains from high-Cd fields. Linear regression (R^2=0.714) indicates a positive significant relationship between total Cd in soils and Cd concentrations in rice grains ($p<0.001$). This increased to R^2=0.918 ($p<0.001$) with the omission of the three fields, F14-F16 (Figure 3.12), with medium- and high-Cd contaminated soils but with lower grain Cd content (0.37, 0.65 and 1.19 mg kg^{-1}) than would be expected from the linear relationship based on all data in Figure 3.12. These fields remained flooded for longer because they are the fields closest to the drainage outlet leading to the canal. Indeed the soil moisture contents for these fields during the harvest periods were slightly higher (22.5, 28.0 and 21.0%) than for the other fields where the moisture content range was 13.6-21.2%. The absence of detectable CdS phases in these soils indicates that differing redox status should have little influence on the availability of soil Cd, but nevertheless there must be some process limiting available Cd within these fields. A Japanese study on a different soil type with very different mineralogy, found that flooding after heading (start flowering) was effective in reducing rice grain Cd content and also limited the accumulation of As (Arao *et al.,* 2009).

Table 3.17 Cd Concentrations in Soil and Rice Plant Components and the Ratio of Grain Cd: Root Cd Expressed as a Percentage (mean ± standard error; n=2)

Contaminated soils		Cd concentrations in soil and parts of rice (mg kg^{-1})					Ratio (%)
		Soil	Root	Straw	Husk	Grain	grain: root
Low-Cd	F1	4.7±0.08	5.8±0.01	0.02±0.00	0.05±0.00	0.07±0.02	1.27
	F7	3.7±0.06	5.4±0.01	0.06±0.03	0.09±0.01	0.12±0.02	2.25
Medium-Cd	F4	16.8±0.47	24.8±0.43	0.27±0.02	0.27±0.00	0.90±0.00	3.61
	F14	35.4±4.34	16.7±0.12	0.14±0.01	0.12±0.00	0.37±0.02	2.24
High-Cd	F10	83.7±0.44	131.5±1.95	2.39±0.17	4.26±0.00	4.03±0.03	3.07
	F18	81.9±4.74	86.1±2.13	3.10±0.49	1.90±0.02	2.77±0.02	3.22

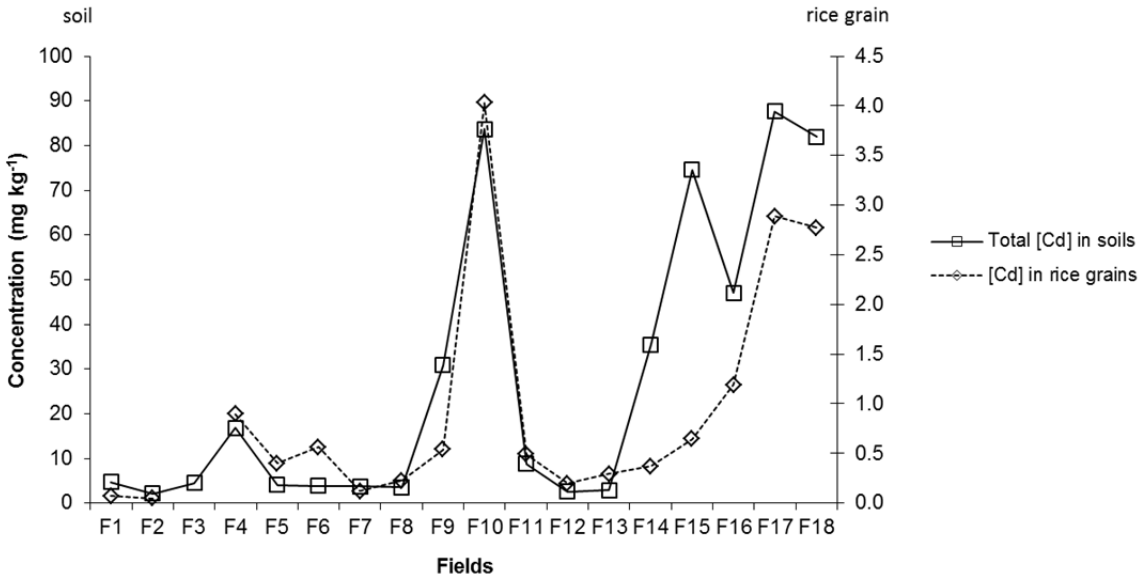


Figure 3.11 Cd concentrations in soils and rice grains from each of the 18 fields

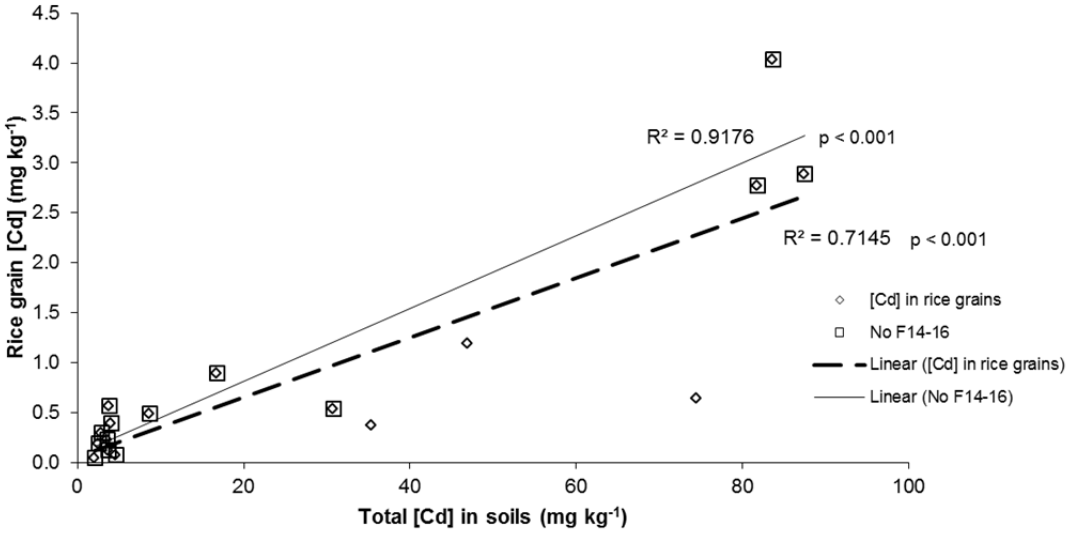


Figure 3.12 Linear regressions for total [Cd] in rice grains (mg kg^{-1}) versus [Cd] in soils collected from the group of 18 fields (F1-18) and from 15 fields (F14-F16 removed)

In this study, the initial steps of the Tessier and BCR sequential exchange methods both generated results that had significant positive relationships with grain Cd ($p<0.001$ and $p=0.001$, respectively; Figure 3.13) even for F14, indicating their utility for broadly estimating available Cd in these soils and Cd absorption into food stuffs and human exposure via food intake.

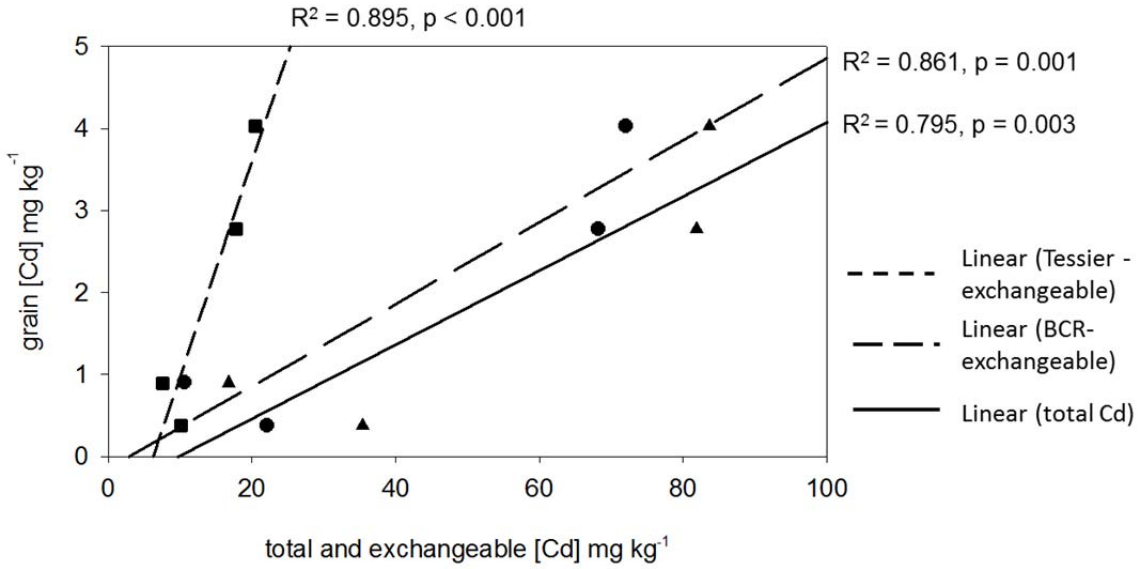


Figure 3.13 Linear regressions for total (▲; ——), BCR-exchangeable (●; – –) and Tessier-exchangeable (■; ---) [Cd] versus rice grain [Cd] for medium (F4, F14)- and high (F10, F18)-Cd contaminated fields

3.3 Conclusions

The creek and canal waters were slightly alkaline with mean pH value of ~7.8 over the entire sampling period. The redox measurements on these waters indicated that the waters were oxic in the dry period but became progressively sub-oxic as the creek and canal flooded through to the harvest period. The canal waters in the harvest period had the lowest E_h values.

Dissolved (<0.2 μm) Cd concentrations in the creek and canal waters were highest during the flood period and decreased with increasing distance from Cr1 towards the paddy fields. During the flood period, concentrations of Cd associated with suspended particulate matter were typically higher than those in the dissolved phase. Indeed, >89% of the total Cd in the creek and canal waters was present in the

particulate fraction (>0.2 μm). Importantly, the same trend with distance was observed for the suspended particulates as was described above for the dissolved phase, indicating that much of the contamination from the mining area is being removed by sedimentation in the creek and canal before the paddy fields.

In the dry period, it was shown that Cd concentrations were higher behind the check dams, especially between the main check dam and the small check dam. This is consistent with settling out of suspended particulate material in the canal which was shallower than the main creek.

The canal sediments comprised predominantly <53 μm particles containing calcite, dolomite and phyllosilicates but not hemimorphite or smithsonite. Here, sequential extraction showed that Cd was associated with both clay particles and carbonate/clay aggregates. Suspended particles did not contain calcite or dolomite but did contain clay minerals, especially illite. Therefore, since >80% of the Cd in water samples was associated with these suspended particles , it is concluded that fine clay particles bearing Cd are being transferred onto the paddy fields, especially during the wet season. Cadmium concentrations in the paddy field topsoils did not decrease with increasing distance from the creek. Instead, there was a strong influence of topography and man-made manipulations. Specifically, the highest Cd concentrations were observed in the lowest lying fields including those which had received topsoil excavated from the fields closer to the source of contamination and/or dredging material from the canal.

The fields with the highest topsoil Cd concentrations also had the highest concentrations of Zn, Mg, Ca, Pb and S, providing a clear link to the source material since the ore-proxy material obtained from near the Zn mining area contained calcite ($CaCO_3$), dolomite ($CaMgCO_3$) as well as hemimorphite ($Zn_4(Si_2O_7)(OH)_2.2H_2O$) and smithsonite ($ZnCO_3$) and it is also known that the primary ore body contains sulfides of both Zn and Pb.

SEM-EDX analysis of the canal sediment, Ca3, and topsoil from the heavily contaminated field, F10, identified two forms of Cd: (i) Cd in association with clay minerals and (ii) Cd in the form of mixed Cd-Ca carbonates. Association of Cd with both clay and with carbonate particles within the soils is consistent with the additional transfer of canal sediment during dredging activities.

Additional evidence for the associations of Cd within the paddy soils was obtained using sequential extraction. The results showed that there was a high proportion of BCR1-extractable Cd in low-, medium- and high-contaminated paddy soils. The first two steps of the Tessier sequential extraction method revealed a difference between the medium- and high-contaminated soils suggesting that, since similar concentrations of Tessier-extractable Cd were present in both, the greater contamination in the latter group was again due to the deposition of greater quantities of canal sediment bearing Cd-carbonate phases during dredging activities.

Analysis of rice from 18 paddy fields showed that there was a significant positive correlation between rice grain Cd and total soil Cd concentrations, primarily due to the prevalence of easily (BCR1 and T1) extractable Cd within the soil.

Since the Cd concentrations in rice grains from the medium and high Cd contaminated fields were significantly above the recommended safe limit of 0.4 mg kg^{-1}, the factors controlling Cd bioavailability in these Thai soils, and measures to restrict it, required fuller investigation. Chapter 4 focuses on additional methods to evaluate bioavailability in Cd soils with varying extents of contamination while Chapter 5 explores a potential remediation strategy which would be applicable in rural areas of Thailand.

References

Akkajita, P. and C. Tongcumpou. (2010). Fractionation of metals in cadmium contaminated soil: relation and effect on bioavailable cadmium. *Geoderma* 156(3-4): 126-132.

Alloway, B. J. (ed.) 1995. Heavy metals in soils. London: Chapman and Hall. 368 pp.

Arao, T., A. Kawasaki, K. Baba, S. Mori, S. Matsumoto. (2009) Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. *Environmental Science and Technology* 43(24): 9361–9367.

Chantana, P., S. Wittaya, M. Nishijo, R. Werawan and I. Thawangon. (2007) Health risk management for cadmium contamination in Thailand: are challenged overcome? http://www.medassocthaithai.org/Journal/files/Vol90_No.1_143_8163.pdf.

Department of Sustainable Natural Resources. (2010) Soil survey standard test method particle size analysis. http://www.environment.nsw.gov.au/resources/soils/testmethods/psa.pdf.

FAO/WHO. (2006) Codex alimentarius commission: report of the twenty-ninth session Geneva, 3-7 July 2006. Joint FAO/WHO standard programme.

Huang, S.S., Q.L. Liao, M. Hua, X.M. Wu, K.S. Bi, C.Y. Yan, B. Chen and X.Y. Zhang. (2007) Survey of heavy metal pollution and assessment of agricultural soil in Yangzhong district, Jiangsu Province, China. *Chemosphere* 67(11): 2148-2155.

Jo, I.S. and M.H. Koh. (2004) Chemical change in agricultural soils of Korea: date review and suggested countermeasures. *Environmental Geochemistry and Health* 26(2-3): 105-107.

Jung, M.C. and I. Thornton. (1996) Heavy metal contamination of soils and plants in the vicinity of a lead-zinc mine, Korea. *Applied Geochemistry* 11(1-2): 53-59.

Khaokaew, S., R. Chaney, G. Landrot, M. Ginder-Vogel and D. Sparks. (2011) Speciation and release kinetics of cadmium in an alkaline and acidic paddy soils under various flooding periods and drain conditions. *Environmental Science and Technology* 45(10): 4249-4255.

Kosolsaksakul, P., J.G. Farmer, I.W. Oliver, M.C. Graham. (2014) Geochemical associations and availability of cadmium (Cd) in a paddy field system, northwestern Thailand. *Environmental Pollution* 187: 153-161.

Lertlakanawong, P. (ed.) (2005) Summary report: Participatory rural appraisal on Mae Tao sub watershed program, submitted to Thai National Health Foundation. Bangkok: Local Development Institute pp. 51. (in Thai)

McLaughlin, M.J., D.R. Parker and J.M. Clarke. (1999) Metals and micronutrients - food safety issues. *Field Crops Research* 60(1-2): 143-163.

Padaeng Industry Public company limited. (2004) Annual report 2004. pp.13.

Padungtod, C., W. Swaddiwudhipong, M. Nishijo, W. Ruangyuttikarn, and T. Inud. (2006) *Health Risk Management for Cadmium Contamination in Thailand: Are Challenges Overcome?*. Forum V side event: Health and environmental concerns associated with heavy metals; global needs for further action? Budapest Conference on Heavy Metals 23 September 2006.

Paijitprapapon, A., K. Udomtanateera, O. Udomtanateera, V. Chariyapisuthi, T. Chengsuksawat. (2006) Environmental investigation for causality and remediation of cadmium contaminated soils in Tak Province, Thailand. Chinese Journal of *Geochemistry* 25(1 Supplement): 253-254.

Pan, J., J.A. Plant, N. Voulvoulis, C.J. Oates and C. Ihlenfeld. (2010) Cadmium levels in Europe: implications for human health. *Environmental Geochemistry and Health* 32(1): 1–12.

Pongsakul, P. and S. Attajarusit. (1999) Assessment of heavy metals in soils. *Thai Journal of Soil Fertility* 21: 71-82.

Prakongkep, N., A. Suddhiprakarn, I. Kheoruenromne, M. Smirk and R.J. Gilkes (2008) The geochemistry of Thai paddy soils. *Geoderma* 144(1-2): 310–324.

RTC. (2010) Certificate of analysis: Natural matrix certified reference material Catalogue No: CRM051-050 Lot No: 010403.

Simmons, R.W., A.D. Noble, P. Pongsakul, O. Sukreeyapongse and N. Chinabut. (2008) Analysis of field-moist Cd contaminated paddy soils during rice grain fill allows reliable prediction of grain Cd levels. *Plant and Soil* 302(1-2): 125–137.

Simmons, R.W., P. Pongsakul,D. Saiyasitpanich, S. Klinhoklap, (2005) Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice downstream of a zinc mineralized area in Thailand: implication for public health. *Environmental Geochemistry and Health* 27(5-6): 501-511.

Swaddiwudhipong, W., P. Limpatanachote, P. Mahasakpan, S. Krintratun , C. Padungtod. (2007) Cadmium-exposed population in Mae Sot District, Tak Province: 1. Prevalence of high urinary cadmium levels in the adults. *Journal of the Medical Association of Thailand* 90(1): 143-148.

Swaddiwudhipong, W., P. Limpatanachote , P. Mahasakpan, S. Krintratun, S. (2012) Progress in cadmium-related health effects in persons with high environmental exposure in northwestern Thailand: a five-year follow-up. *Environmental Research* 112: 194-198.

Tessier. A., P.G.C. Campbell, M. Bisson. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* 51(7): 844-851.

Thai Meteorological Department. (2009) Climate Statistics Data of Mae Sot District, Thailand (1977-2008).

Ure, A.M., Ph. Quevauvillerb, H. Muntauc and B. Griepinkb. (1993) Speciation of heavy-metals in soils and sediments - an account of the improvement and harmonization of extraction techniques undertaken under the auspices of the

BCR of the Commission-of-the- European-Communities. *International Journal of Environmental Analytical Chemistry* 51(1-4): 135-151.

USEPA. (1996) Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3052.pdf .

USEPA. (2007) Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils, and oils. http://www.caslab.com/EPA-Methods/PDF/EPA-Method-3051A.pdf.

Walker, B. L. (2010) Toxic archipelago: a history of industrial disease in Japan. Seattle: The University of Washington Press. pp.284.

Yafa, C., S. Srithongtim, P. Phukphatthanachai, A. Thiparuk, N. Sudsiri, L. Rojanapantip. (2010) New reference material for analysis of elements in glutinous rice produced at the National Institute of Metrology (Thailand). *Accreditation and Quality Assurance* 15: 223-231.

Zarcinas, B.A, P. Pongsakul, M.J. McLaughlin and C. Cozens. (2004) Heavy metals in soils and crops in Southeast Asia 2. Thailand. *Environmental Geochemistry and Health* 26: 359-371.

Zhai, L., X. Liao, T. Chen, X. Yan, H. Xie, B. Wu, and L. Wang. (2008) Regional assessment of cadmium pollution in agricultural lands and the potential health risk related to intensive mining activities: a case study in Chenzhou city, China. *Journal of Environmental Sciences* 20(6): 696-703.

Zhao, H., B. Xia, C. Fan, P. Zhao and S. Shen. (2012) Human health risk from soil heavy metal contamination under different land uses near Dabaoshan Mine, Southern China. *Science of the Total Environment* 417-418: 45-54.

Chapter 4 Bioavailability of Cd Using Stable Isotope Exchange

In the previous chapter, the bioavailability of Cd was assessed using the BCR sequential extraction method and the first two steps of the Tessier extraction scheme. It has been suggested, however, that isotope exchange is a more sensitive means of measuring metal lability. This chapter focuses on the use of a stable isotope technique as an alternative way to assess Cd bioavailability and a comparison between all three approaches will be made.

4.1 Stable Isotope Exchange (SIE)

The bioavailable fraction of an element present in soil, also referred to as the labile fraction (e.g. Tiller *et al.*, 1972; Nakhone and Young, 1993) is that fraction available to organisms. Because contamination of food chains is a major concern in regard to metal pollution in soils, estimation or approximation of the plant available (or phytoavailable) content in particular has been the focus of much research (e.g. Echavarria *et al*., 1998; Brun *et al*., 2001; Li *et a*l., 2004: Oliver *et al*., 2006).

Conventional methods such as selective extraction, e.g. to give the ion exchangeable or organic complexation forming fractions, and sequential extraction, e.g. to give exchangeable, oxidisable and reducible fractions, have been commonly used because (i) they are less time-consuming than other methods; or (ii) they may be assured by external bodies such as the Commission of the European Communities. These methods, however, have received considerable criticism in the literature. For example, extractants may mobilise non-specific fractions of soil metals, while variation in the concentration of the extracting reagent and/or the soil: solution ratio can result in differing quantities of metal being extracted (de Vries, 1983; Fujii and Corey, 1986). Also, metals released by one reagent may be re-adsorbed to remaining

solid phases during extractions and thus the available fraction may be underestimated (Bermond, 2001). Other researchers have noted that the order of reagents applied in a sequential extraction scheme can affect the amount of metal identified as being associated with a particular fraction (e.g. McGrath and Cegarra, 1992; see also Young *et al.*, 2006). Such issues with these techniques have led some to conclude that the most rigorous way of assessing the lability of metals in soil is probably by direct measurement via isotope exchange (Nakone and Young, 1993; Huang *et al*., 2011; Smolders *et al*., 1999). As outlined by Degryse *et al*. (2011), isotopic exchange methods discriminate between isotopically exchangeable ('labile') and non-exchangeable ('non-labile') metal pools in soils and involve adding a small amount of an isotopic tracer of the analyte to the soil and determining the degree to which the native element within the soil can exchange between solid and solution phases.

Isotope exchange analysis has been used in the study of chemical processes in many fields of research (Meija and Mester, 2008), e.g. nutrient transport and metabolism (Mann, 1989; Flynn, 1998); drug absorption (Aggarwal *et al*., 1993); availability of elements in soil ecosystems (Booth *et al.,* 2005; Hamon *et al.,* 1997; Nakone and Young, 1993). Stable and/or radioactive isotopes of a number of elements, e.g. Co, Cu, Cd, Ni, P, Pb, S and Zn, have been used to investigate their availability in soils. In the past, highly sensitive spectrometric techniques for measuring stable isotope masses with high precision and accuracy were not widely available and so radiometric methods were more commonly used. However, the development of ICP-MS and Thermal Ionization Mass Spectrometry (TIMS) instruments along with decreasing purchase costs have made such high sensitivity stable isotopic analyses much cheaper and more widespread. Consequently, the stable isotope exchange approach, developed from methods involving radioactive isotope analysis, e.g. the work of Nakhone and Young (1993) and Tiller *et al*. (1972), is now favoured. The main reasons for this are (i) the very short half-lifes of some isotopes, e.g. ^{64}Cu (~12.4 h), and more importantly (ii) concerns about the safety of handling and storing radioactive isotopes. Moreover, the developed stable isotope methods have been shown to give similar results (Sterckeman *et al.,* 2009; Nolan *et al.,* 2004) to the earlier radioactive methods.

Typically, stable isotope techniques involve the addition of an isotope which is present in very low proportions in the natural form of the element as a tracer (or spike) into a soil suspension where the aqueous phase contains electrolytes such as $CaCl_2$ and $MgCl_2$. The added spike is allowed to exchange with the natural isotope of the same element until equilibrium has been reached (Figure 4.1). For many elements, this isotopic equilibrium is nearly reached within 24 h, although some continued slow exchange continues for longer periods (Huang *et al*., 2011; McLaren and Crawford, 1974; Tongtavee *et al*., 2005; Xie *et al*., 2012). It is important to note that with the isotope technique, the amount of element added must be small (both in absolute terms and as a proportion of the total present in the sample), ensuring that the equilibrium between solid and solution is not disturbed by the spiking process (Degryse *et al*., 2011). Isotopic exchange is, however, relatively robust to changes in the solid: liquid ratio and electrolyte composition (Young *et al*., 2006).

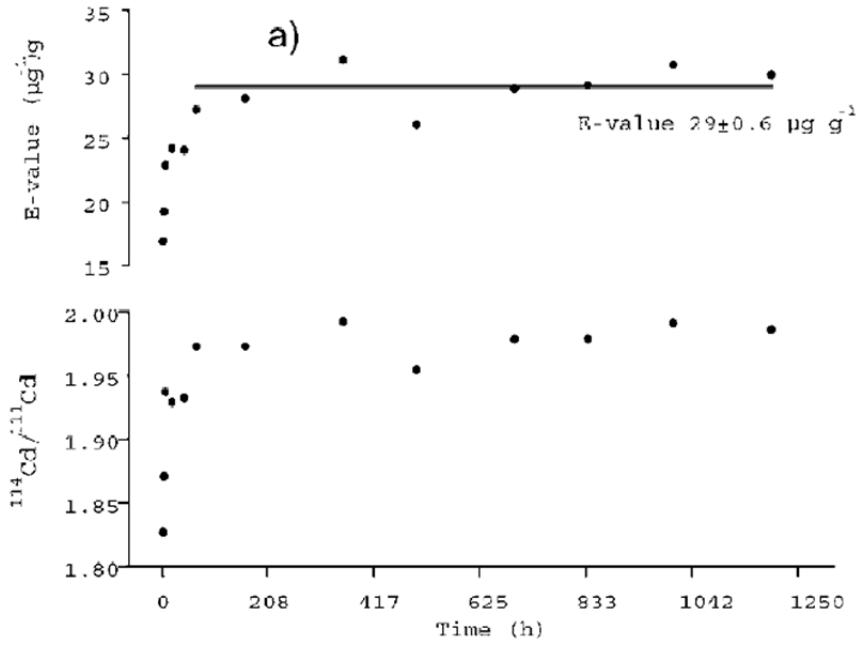


Figure 4.1 Demonstration of the time taken to reach a status of equilibrium for $^{114}Cd/^{111}Cd$ isotope ratios and resulting calculated E values (Ayoub *et al.*, 2003)

At equilibrium, the ratio of spiked and natural isotopes of the element in the supernatant are quantified by ICP-MS and the exchangeable (E) value is calculated using that ratio and the amount of spiked isotope added, the isotopic composition of the spike and the known or experimentally determined isotopic composition of the natural form of the element in the sample (Huang *et al.*, 2011). Following the same principle, the isotopically exchangeable pool can be alternatively determined by growing a plant in spiked soil and measuring the same isotopic ratio in the plant tissue. This has become known as the L value and has the benefit of including a biotic aspect to the measurement (Tiller *et al*., 1972; Hamon *et al*., 1997; Oliver *et al*., 2006). Importantly, many research papers have demonstrated close agreement between E and L value measurements when conducted on the same soils (Ayoub *et al.*, 2003; Oliver *et al*., 2006; Wendling *et al*., 2008). Some studies have, however, identified circumstances where E and L value measurements differed, with the discrepancies possibly being attributable to root exudates altering the lability of metals in the rhizosphere (e.g. Hutchison, 2000). Primarily in relation to Zn measurements, discrepancies and other limitations have also been linked to fixation of the added isotopic tracer to soil solids and increased sorbing effects of colloids in high pH soils (e.g. Tiller 1972: Sinaj *et al*., 2004 Lombi *et al*., 2003; Tongtavee *et al.*, 2005).

Even with these potential limitations, the demonstrated robustness of isotopic exchange techniques has led to them being considered as one of the most reliable methods for assessing bioavailability of metals in soils. A limited number of investigations comparing isotopic exchange techniques with assessments based on sequential or selective extractions do exist and these have produced varied results; some have found a reasonable agreement between the approaches while others found that chemical extractants poorly simulated isotopic exchange (e.g. Degryse *et al*., 2011; Young *et al*., 2000; Young *et al*., 2006). Whether or not chemical extracts can approximate bioavailability assessments made with isotopic exchange may depend on soil properties and contamination history of a site and therefore further comparative studies are needed in order to determine when and in what circumstances the cheap and rapid option of chemical extraction can be used in place

of isotopic exchange. Evaluation of isotopic exchange techniques and comparison with extraction methods in assessing the availability of Cd in the contaminated Thai soils is thus the focus of this chapter.

An overarching objective of this PhD was to establish links between the solid phase Cd speciation and Cd availability to rice plants by using bioavailability assessing methods e.g. Tessier sequential extraction, BRC extraction and a stable isotope exchange technique. The specific objectives of this chapter were (1) to develop and use a stable isotope exchange technique to determine labile Cd in Cd-contaminated soils and (2) to compare the stable isotope exchange technique with others measurements and methods, i.e. total Cd, BCR1 and Tessier-exchangeable Cd.

4.2 Methods

4.2.1 Stable Isotope Exchange Using ^{111}Cd

The experimental method used in this study follows that described in Sterckeman *et al.* (2009). An enriched ^{111}Cd solution, in 2% HNO_3 (IES-Cd111, Innovative Solutions in Chemistry; ^{106}Cd 0.008%, ^{108}Cd 0.008%, ^{110}Cd 0.351%, 111Cd 96.167%, ^{112}Cd 2.004%, ^{113}Cd 0.487%, ^{114}Cd 0.867% and ^{116}Cd 0.108%) was selected as the spike isotope and ^{114}Cd, the most abundant naturally occurring isotope (28.73% abundance), was used as the other isotope in calculations of the isotopic ratio, i.e. ^{114}Cd/^{111}Cd. The spike solution was prepared such that the added Cd represented ~0.5-1% of the total Cd already present in the contaminated soils, thus meeting the requirement for reliable isotopic exchange studies that the added spike does not disturb the equilibrium established between solid and solution phases (as discussed above, see also Degryse *et al.*, 2011; Young *et al.*, 2006). Firstly, a 20-fold dilution of the IES-Cd111 solution (10.224±0.029 mg kg^{-1} as supplied) using 2% v/v Aristar nitric acid was performed. Then, ~1.4 ml of spike solution (~0.7 µg Cd) was

added to a suspension of ~1 g soil in ~20 ml electrolyte (0.01 M $MgCl_2$). The specific details relating to these experiments follow in the paragraph below.

Low Cd- (F1 and F7), medium Cd- (F4 and F14) and high Cd- (F10 and F 18) contaminated soils (see Chapter 3.2.2) were used for the stable isotope exchange experiments. About 1 g of each sample soil (in duplicate) was accurately weighed into a 50 ml centrifuge tube to which 20 ml of 0.01 M $MgCl_2$ was added. The tube was shaken end-over-end for 2 days in order to thoroughly mix the soil and electrolyte solution. After that, 1.4 ml of the diluted ^{111}Cd spike solution was added followed by 60 µL of chloroform to avoid the effect of any microorganism activity in the tubes. The suspensions were shaken for one week to ensure that the exchange process had reached equilibrium. Thereafter, the tubes were centrifuged at 7000 rpm for 15 minutes (6793 *g*) and the supernatant solution was passed through a 0.22 µm syringe filter (33 mm Millex® Syringe Filters) in preparation for ICP-MS analysis. The ICP-MS conditions are shown in Table 4.1.

Table 4.1 The Conditions Used During ICP-MS Analysis of the Solutions Obtained after Isotopic Exchange with Selected Paddy Soils

Instrument	**Parameters**
RF power	1540 W
Reflected power	1 W
Argon gas carrier flow	0.82 L min^{-1}
Argon gas make-up flow	0.21 L min^{-1}
Nebuliser up-take rate	0.2 mL min^{-1} (0.06 rps)
Analyser pressure (vacuum)	3×10^{-6} Pa
IF/BK pressure (vacuum)	8.5×10^{-1} Pa
Rinse speed (rinse port)	0.3 rps
Between sample rinse time, rinse vial	40 sec
Rinse speed (rinse vial)	0.1 rps
Between sample rinse time, rinse port	10 sec
Number of replicates	3

The E value calculation involved the use of Equation 4.1, the derivation of which has been explained previously (e.g. Nolan *et al.*, 2004 and Oliver *et al.*, 2006).

$$E\ value\ (mg\ kg^{-1}) = R\ \times \frac{AWnat}{AW(^{111}Cd)} \times \frac{IRsp - IRmes}{IRmes - IRnat} \times\ (IRnat + 1)$$

- Equation 4.1

where

E value	=	isotopically exchangeable Cd (mg kg^{-1}),
R	=	total concentration of ^{111}Cd in the spike (µg) / soil weight (g),
AW(^{111}Cd)	=	atomic weight of ^{111}Cd isotope,
AWnat	=	atomic weight of natural Cd (112.411)
IRnat	=	the ^{114}Cd/^{111}Cd ratio based on natural abundances of these isotopes,
IRsp	=	the ^{114}Cd/^{111}Cd ratio in the spike solution
IRmeas	=	measured ^{114}Cd/^{111}Cd ratio in the soil solution after equilibration.

Quality Control: To have confidence in the isotope ratio results obtained by ICP-MS analysis, the variability of ^{114}Cd/^{111}Cd in standard solutions was checked throughout each run. Figure 4.2 shows the relationship between counts obtained by ICP-MS and the concentration of each standard solution (0.5-100 µg L^{-1}) for each of the four main naturally occurring Cd isotopes (^{106}Cd, ^{108}Cd, ^{111}Cd and ^{114}Cd). There was a high degree of linearity in the instrumental response over this concentration range and R^2 values of 0.999 or better were obtained for each line shown in Figure 4.2. The ratio of the natural ^{114}Cd/^{111}Cd in unspiked soil samples obtained during this study was 2.34±0.005 (n=11). This compares well with the theoretical ratio value of 2.25

(based on isotopic abundance) and also demonstrates that between-run variability was low. Moreover, the $^{114}Cd/^{111}Cd$ ratio in the CRM SRM1643, which had a certified Cd concentration of 6.568±0.073 mg kg^{-1}, had a mean $^{114}Cd/^{111}Cd$ ratio of 2.33±0.007 (n=10), in good agreement with the values obtained for the soil.

To establish within-run variability, the $^{114}Cd/^{111}Cd$ ratio values were obtained for the 5 and 10 µg L^{-1} standard solutions which were run after every 5-6 samples. The values of 2.32±0.004 (5 µg L^{-1} standard; n=3) and 2.35±0.003 (10 µg L^{-1}; n=4) were in close agreement with those obtained for the soil samples and the low standard deviations indicated that the ICP-MS remained stable throughout the run (Figure 4.3). The $^{114}Cd/^{111}Cd$ ratio of the ^{111}Cd spiked blanks (i.e. no soil, just 20 ml of 0.01 M $MgCl_2$; in duplicate) were determined in two separate batches as 0.01291±0.0002 and 0.01183±0.0003. This was only slightly higher than the calculated $^{114}Cd/^{111}Cd$ ratio of the pure enriched ^{111}Cd spike (IES-Cd111, calculated ratio 0.009016).

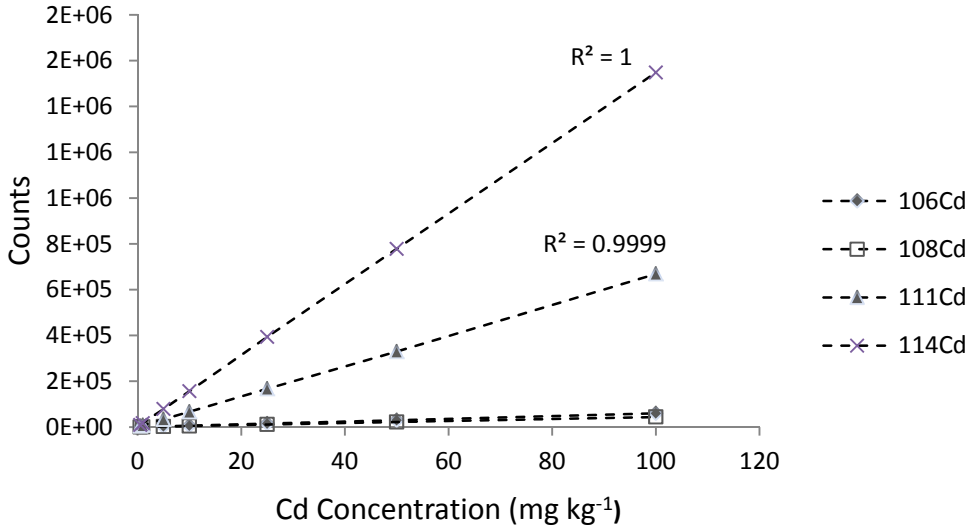


Figure 4.2 The linearity of ICP-MS response for the four main naturally occurring Cd isotopes in standard solutions

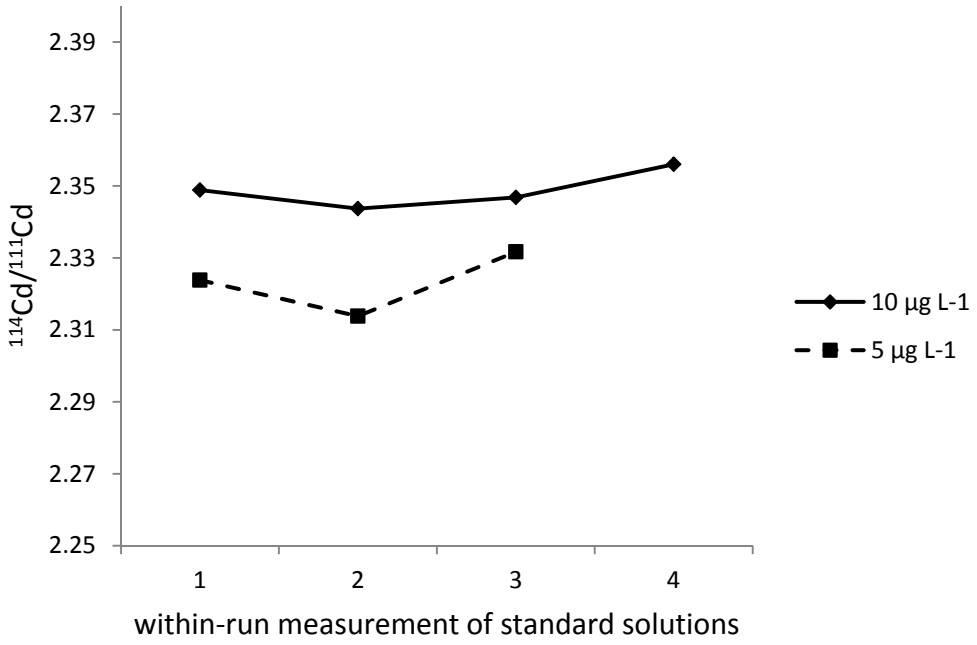


Figure 4.3 Illustration of the within-run variability in the $^{114}Cd/^{111}Cd$ ratio obtained for 5 µg L^{-1} and 10 µg L^{-1} standard solutions

4.2.2 Comparisons with BCR and Tessier Sequential Extractions

As previously discussed, the BCR sequential scheme has been widely used to distinguish between different associations of elements including Cd within soils. In particular, the first step, BCR1 (exchangeable) has been used to estimate elemental bioavailability. In this chapter the results from the earlier BCR work (specifically BCR1 for the medium- and high-Cd contaminated soils, Chapter 3) are used to compare with and discuss the results of the SIE technique.

The first two steps of the Tessier sequential extraction scheme used in this section were slightly adapted from those described in Tessier *et al.* (1979) (see Chapter 3.2.3). The first step involves release of exchangeable forms of the element and the second step involves extraction of carbonate bound forms of the element. This

method was conducted in Chapter 3 and the results will be used to compare with the SIE results.

4.3 Results and Discussion

4.3.1 E values for Selected Paddy Field Soil Samples

The E values, a measure of the isotopically labile fraction of Cd for the selected soil samples, were in the range 0.75-19.0 mg kg^{-1} and there was extremely good agreement between the duplicates for each soil sample (Table 4.2). These values corresponded to 20.2-41.3% of the total Cd in the soils. In terms of percentage of total Cd, the results obtained for the low-Cd soils varied by a factor of two (41.3% and 20.2% for F1 and F7, respectively). In contrast, the medium-Cd soils (F4 and F14) had consistently high proportions of labile Cd (~41%), which were almost double those for the high-Cd soils (i.e. ~22% for F10 and F18, Table 4.2). Thus, unexpectedly, there was proportionally lower Cd lability in the more highly contaminated soils (where concentrations were >50 mg kg^{-1}). However, in mg kg^{-1} terms (i.e. not as a fraction of total Cd), the labile Cd content was highest in the high-Cd soils (i.e. 18 to 19 mg kg^{-1} in high Cd soils vs ~7 to 14.5 mg kg^{-1} in medium-Cd soils; Table 4.2).

Table 4.2 E values of Low-, Medium- and High-Cd Contaminated Soils (mean of duplicate ± standard error)

Source	Fields	E values ($mg\ kg^{-1}$)	Lability expressed as % of total Cd
Low-Cd contaminated soils	F1	1.93±0.0004	41.3±0.07
	F7	0.75±0.0002	20.2±0.06
Medium-Cd contaminated soils	F4	6.92±0.07	41.3±0.40
	F14	14.5±0.16	41.0±0.44
High-Cd contaminated soils	F10	19.0±0.14	22.7±0.17
	F18	18.0±0.62	22.0±0.76

The Cd E values of these Thai soils (0.75-19.0 mg kg-1; 20.2-41.3%) were lower in $mg\ kg^{-1}$ terms but higher in terms of percentage of total Cd than those determined using a radio-isotope method in a UK soil from Derbyshire that had been historically contaminated by Pb/Zn mine spoil (19.6–52.5 $mg\ kg^{-1}$; 6.8– 15.1%; total Cd 130-770 $mg\ kg^{-1}$; Hutchinson *et al.,* 2000). Two French soils contaminated by atmospheric fallout from a Pb/Zn smelter (total Cd ~20 $mg\ kg^{-1}$) were found to have E values in the range 6.9-16.4 $mg\ kg^{-1}$ (36.3-78.5% of total Cd) (Sterckeman *et al.,* 2009) and thus exhibited a similar range to these Thai soils. Interestingly, the French study (Sterckeman *et al.,* 2009) also examined a number of soils described as having developed from calcareous parent materials enriched in geogenic Cd (total Cd 3.1-22.9 $mg\ kg^{-1}$), which may be comparable to the carbonate rich Thai paddy soils investigated in the current study. Those naturally geochemically enriched soils were found to have E values 0.1-10.6 $mg\ kg^{-1}$ representing 1.1-59.2% of total Cd (Sterckeman *et al.,* 2009) and thus did compare reasonably with the Thai soils being examined here.

4.3.2 BCR1, Tessier Exchangeable (T1) and Tessier Carbonate Bound (T2) Compared with E values for Cd in Selected Paddy Field Soils

As detailed in Chapter 3 (section 3.2.3), BCR1 (0.11 M acetic acid extract) nominally releases water soluble, exchangeable and carbonate-bound forms of Cd from the soil. The BCR1 values were 1.97±0.05 (F1) and 0.64±0.01 (F7) mg kg^{-1} in low-Cd contaminated soils, 10.6±0.5 (F4) and 22.1±0.23 (F14) mg kg^{-1} in medium-Cd contaminated soils, and 72.0±2.1 (F10) and 68.2±0.94 (F18) mg kg^{-1} in high-Cd contaminated soils (Table 3.14). As a percentage of total soil Cd, Cd in BCR1 increased linearly (R^2=0.99) with increasing extent of soil contamination (cf values of 42.2% and 17.3% in low-Cd contaminated soils, 63.2 and 62.4% in medium-Cd contaminated, 86.1 and 83.3% in high Cd contaminated soils). For medium and high Cd contaminated soils, the E values obtained from the experiments described in section 4.3.1 were lower than the amount extracted in BCR1 (Figure 4.4). In addition, there was only a moderate increase in E value from medium to high Cd-contaminated soils, particularly in relation to percentage of total Cd. The relationship between the BCR1 and E values, considering all soils tested, was examined and a logarithmic trend line was found to fit the data best, having a R^2=0.972 (Figure 4.5).

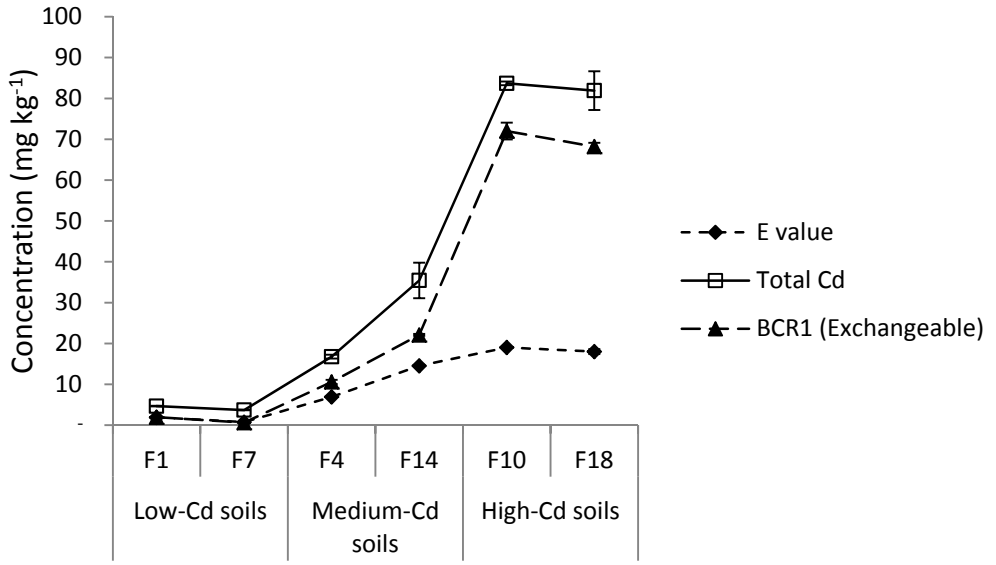


Figure 4.4 Comparison of mean ± standard error of bioavailable Cd as determined by (i) E values and (ii) BCR1 (Exchangeable) extraction, with total Cd concentrations in selected paddy field soils

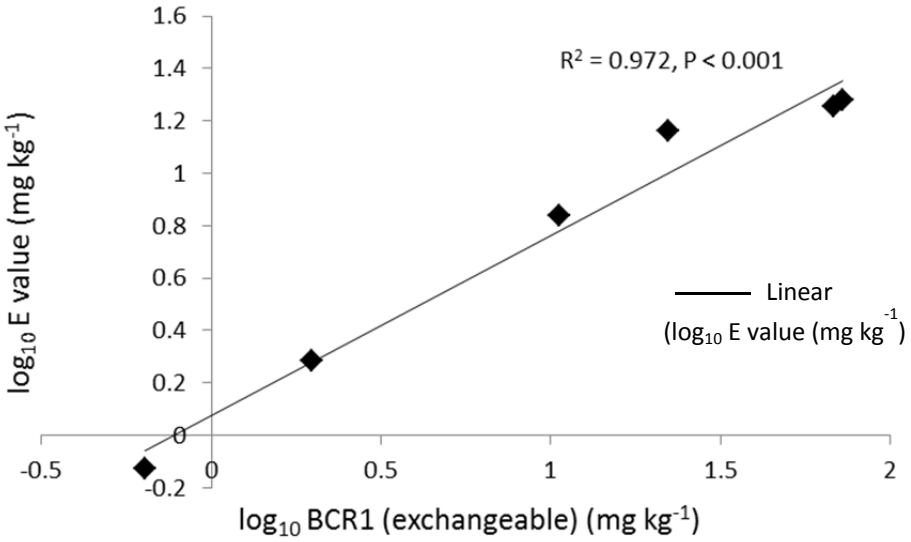


Figure 4.5 Relationship between mean ± standard error of log_{10} E value and log_{10} BCR1 (Exchangeable) extractable Cd in selected paddy field soils

With most of the E values being lower than the BCR1-extracted Cd, it was anticipated that the E value-defined labile Cd may approximate a portion within the forms extracted by the BCR1 reagent. The first steps of the Tessier method are intended to separately extract the exchangeable form and carbonate bound form (both of which are targeted together by BCR1), hence Tessier 1 (T1) and Tessier 2 (T2) were performed on medium Cd- and high Cd-contaminated soils. It was found that T1 Cd concentrations were 7.58±0.18 (F4) and 10.1±0.06 (F14) mg kg^{-1} in medium-Cd contaminated soils and 20.4±0.27 (F10) and 17.8±0.24 (F18) mg kg^{-1} in high-Cd contaminated soils, while T2 values were 7.62±0.52 (F4) and 16.4±0.38 (F14) mg kg^{-1} in the medium-Cd contaminated soils and 60.6±0.61 (F10) and 58.1±0.33 (F18) mg kg^{-1} in high-Cd contaminated soils (Table 4.3).

Table 4.3 and Figure 4.6 show that, for both medium-Cd and high-Cd contaminated soils, there was generally close agreement between the E values and the results from the first step of the Tessier sequential extraction scheme. This finding was in

agreement with Young *et al.* (2000) who used the radioactive isotope dilution technique; ^{109}Cd was used as a tracer and the sources of Cd were from sewage sludge and zinc-rich mine spoil. Thus it would appear that Cd bioavailability in these soils can be estimated just as effectively via neutral salt solution extraction (such as with 1 M $MgCl_2$ and 1 M $CaCl_2$) as it can be by using the more expensive and time consuming E value method, which is worthy of further investigation (see section 4.3.4).

In addition to the observation that E values and T1 approximated each other, it was also observed that T1+T2 exceeded BCR1 and approximated total Cd in medium and high Cd soils (Figure 4.6). The higher value of T1+T2 compared with BCR1 may be explained by the stronger reagent in T2; 1 M CH_3COONa at pH 4.5 compared with BCR1 reagent 0.11 M CH_3COOH at pH 2.85.

Table 4.3 Comparison of Bioavailable Cd as Determined by (i) E values, (ii) T1 (Exchangeable) and T2 (Carbonate) Extractions, for Medium and High-Cd Soils (mean of duplicate ± standard error)

Source	Fields	E value ($mg\ kg^{-1}$)	T1 ($mg\ kg^{-1}$)	T2 ($mg\ kg^{-1}$)
Medium-Cd contaminated soils	F4	6.92±0.07	7.58±0.18	7.62±0.52
	F14	14.5±0.16	10.1±0.06	16.4±0.38
High-Cd contaminated soils	F10	19.0±0.14	20.4±0.27	60.6±0.61
	F18	18.0±0.62	17.8±0.24	58.1±0.33

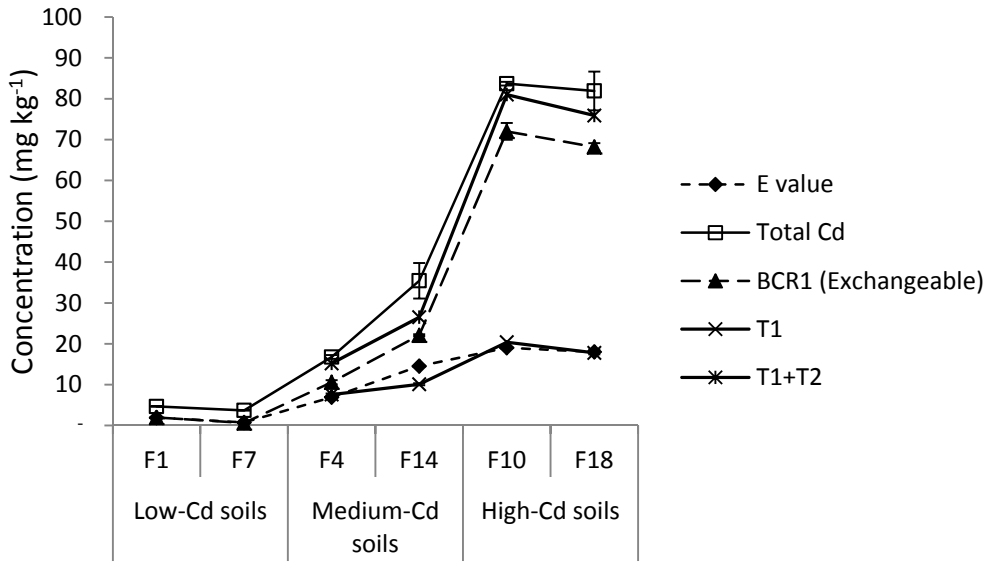


Figure 4.6 Comparison of mean ± standard error of total Cd, E value, BCR1 (exchangeable), Tessier exchangeable forms (T1) and Tessier carbonate-bound (T2) forms of Cd in selected low-, medium- and high-Cd contaminated paddy soils

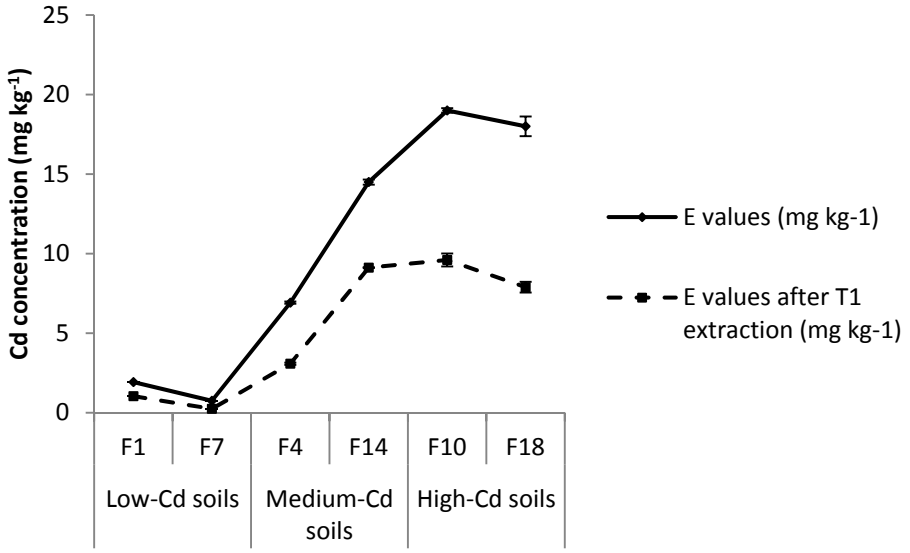
4.3.4 Determination of E value after T1 (exchangeable) Cd has been Removed from Selected Paddy Field Soils

A further experiment was conducted to assess whether or not the T1 (exchangeable) fraction of Cd in the paddy field soils was indeed the same fraction as the pool of labile Cd being accessed by the ^{111}Cd stable isotope added in the spiking experiments (see section 4.3.1). The soil samples from the same low- medium- and high-Cd soils were first extracted using 1 M $MgCl_2$ (pH 7; 2 hours) and after that, on the residue, the E value was determined in the same way as before. Table 4.4 and Figure 4.7(a) indicate that, in all cases, the E value obtained after T1 extraction was lower than that for the non-extracted soil samples. However, there was still a considerable amount of labile Cd as the values obtained after T1 extractions were only ~37–67% lower than the E values for the unextracted soils.

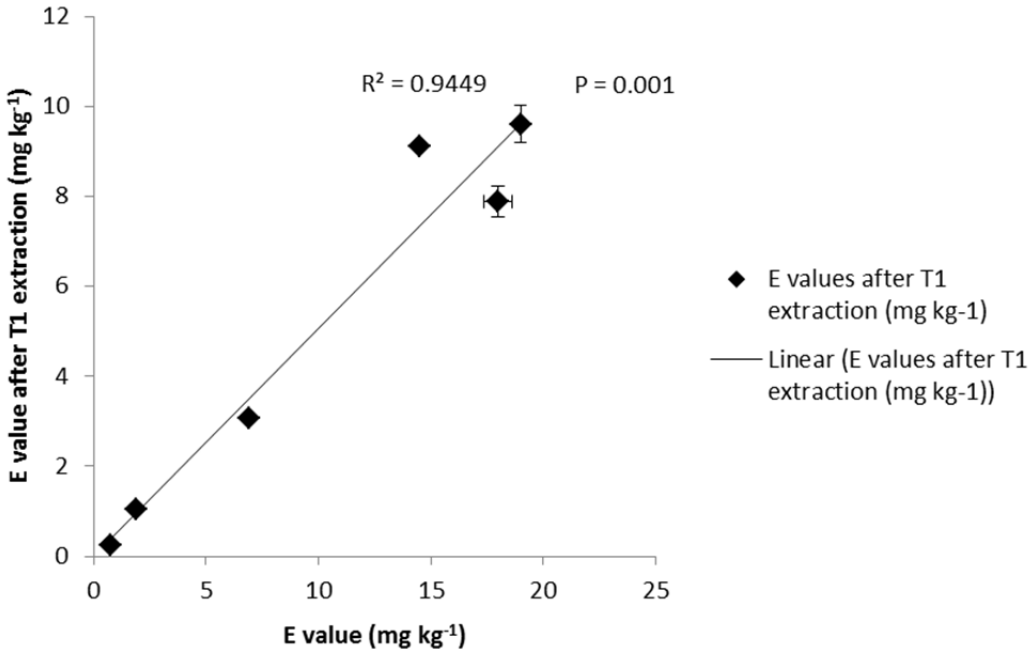
There was a significant positive relationship (R^2=0.944) between the E values before and after T1 extraction (Figure 4.7b). The soil sample from F14 did, however, stand out as being slightly different in that there was less reduction in E value than would be expected based on the results for the rest of the soils. It was also noticeable that the T1-exchangeable Cd in the earlier experiments presented in 4.3 was significantly lower than the E value for this sample. Thus, this may explain why in this soil the E value decreased only by ~37% after T1-exchangeable Cd was removed.

Table 4.4 Comparison of E Value for Total Soil with those Obtained after T1 Extraction of the Same Soils (mean of duplicate ± standard error)

Source	Fields	E values (mg kg^{-1})	E values after T1 extraction (mg kg^{-1})	Reduction in E value after T1 extraction (%)
Low-Cd contaminated soils	F1	1.93±0.004	1.04±0.002	46.0
	F7	0.75±0.002	0.25±0.001	66.6
Medium-Cd contaminated soils	F4	6.92±0.07	3.08±0.07	55.5
	F14	14.5±0.16	9.12±0.01	37.3
High-Cd contaminated soils	F10	19.0±0.14	9.6±0.41	49.5
	F18	18.0±0.62	7.89±0.34	56.1



(a)



(b)

Figure 4.7 (a) The comparison between E values obtained for total soil with E values measured on the same soils after T1 extraction and (b) the linear relationship between these two data sets

The results of this additional study determined that labile Cd defined by the E value method was not the exact same Cd pool as the exchangeable form defined by Tessier 1 (T1), but that about half of labile Cd (as determined by E value measurement) was contributed by T1 (Figure 4.7). This agrees with what Ahnstrom and Parker (2001) had found, insofar as labile Cd was not accountable just from the soluble/exchangeable form (or F1) as defined by the 5 step (i.e. F1-5) study-specific sequential extraction scheme they employed. The soils from three of their four study sites (representing background, sewage and smelter emission contaminated locations) had %E values ranging from 35 to 49% of total Cd, with F1's contribution to E value amounting to only 1-14%. F2 (carbonate bound) was found to hold the majority of Cd in those soils (which had 1.3-6.2 g kg^{-1} carbonate) and this fraction also made the greatest contributions to the isotopically labile Cd (providing 50-66% of E value). F3 (oxidisable components, i.e. organic matter) contributed 32-38% of the isotopically labile Cd. Soil from their fourth site (contaminated by Pb-Zn mine spoil but containing no carbonate) showed a considerably different outcome: Cd in this soil was mostly (82%) associated with the oxidisable fraction (F3), yet only 2% of the Cd in this fraction was isotopically labile. The bulk of the isotopically labile Cd in that mine spoil contaminated soil came from F1 (providing 75% of the E value) (Ahnstrom and Parker 2001).

Reflecting on the current study, a conclusion is drawn that while neutral salt extraction may approximate the E value (or isotopically labile pool) for the soils under examination, the isotopically labile pool can have varying contributions from other fractions that are not directly accessed by the neutral salt extraction and the importance of such contributions may vary among different soils with varying properties (particularly in terms of pH, texture, carbonate content and cation exchange capacity). This is shown conceptually in Figure 4.8. Therefore, although the neutral salt extraction seems to be a good surrogate technique for determining labile Cd in soils (and thus potential uptake by plants), the relationship between the neutral salt and E value needs to be assessed on a much wider variety of soils in

order to be fully quantified. This would also allow determination of the degree to which the neutral salt extraction contributes to the isotopically exchangeable pool.

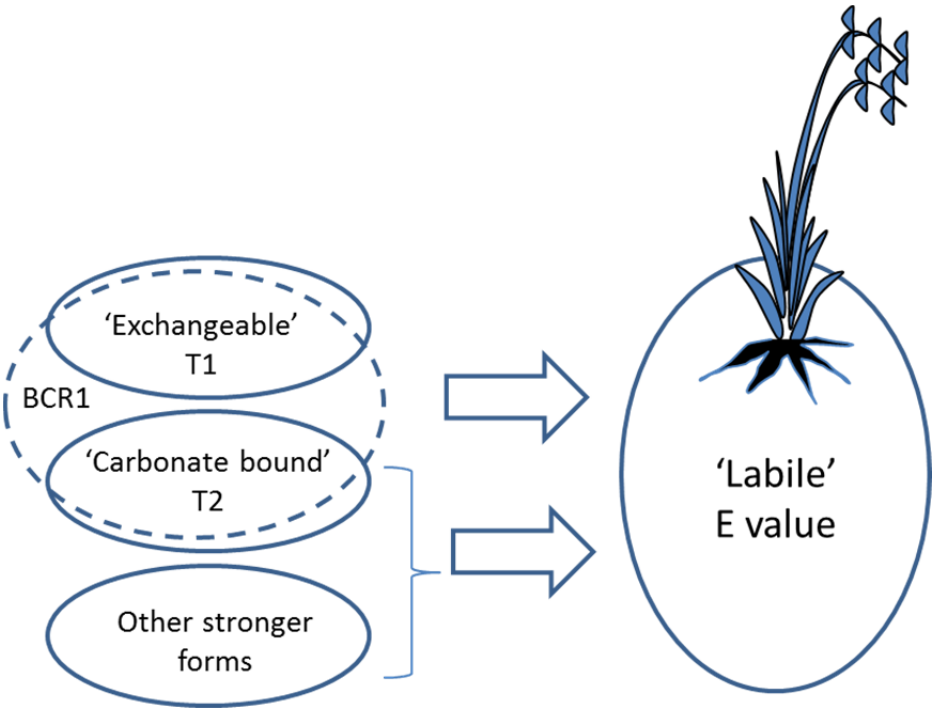


Figure 4.8 Conceptual scheme showing the probable contribution to labile Cd in soils from different soil fractions.

4.3.5 Comparison of Total Cd, E values, BCR1 Cd and T1 Cd with Cd Concentrations in Rice Grains

Figure 4.9(a) shows the significant positive relationship (R^2=0.869; p=0.007) between Cd concentrations in rice grains and total soil Cd obtained for the subset of low-Cd, medium-Cd and high-Cd soils used in this chapter. Figure 4.9(b) shows that the relationship between Cd in rice grains and the E values was weaker (R^2=0.664; p=0.001). This was mainly due to the relatively high E value obtained for F14 soil in

comparison with the particularly low Cd concentration in rice grain for this medium-Cd contaminated field. There was an improved fit when T1 Cd was compared to rice grain Cd concentration but it is clear that this relationship would not pass through the origin (Figure 4.9(c)). The best predictor for Cd concentrations in rice grain was BCR1 Cd with an R^2=0.910 (p=0.003).

Considering the unusualness of the F14 results discussed at various points in the preceding paragraphs and sections, statistical analysis was also conducted on the dataset with F14 temporarily excluded. F14 omission resulted in a very significant positive relationships among Cd in rice and total Cd, E value, BCR1 and T1 (0.938; p=0.007, 0.945; p=0.005, 0.944; p=0.006 and 0.953; p=0.139, respectively) (Figure 4.10). Interestingly, with F14 omitted the highest R^2 value with rice grain Cd was observed for T1 (0.953), as opposed to with BCR1 when F14 was included (0.910; Figure 4.9). However, with the omission of F14 any of the four Cd measurements (total, BCR1, T1 or E value) could be used as an effective predictor of Cd concentration in rice grains.

This finding is in contrast with Simmons *et al*. (2008) who found very poor relationships between rice grain Cd and either total Cd or DTPA-extracted Cd (R^2=0.117 and 0.165, respectively). Those low relationships may have arisen because their study focused only on one field group which had very similar soil mineral compositions and farming management practices and thus provided too narrow a parameter range to identify a relationship.

However, considering the soils tested in the present study, the reason for the difference in F14 needs to be pondered and investigated further because it is possible that other fields in the region may exhibit similar traits. Therefore, if considering all data collected (including F14), the most robust predictor of rice grain Cd would be identified as BCR1.

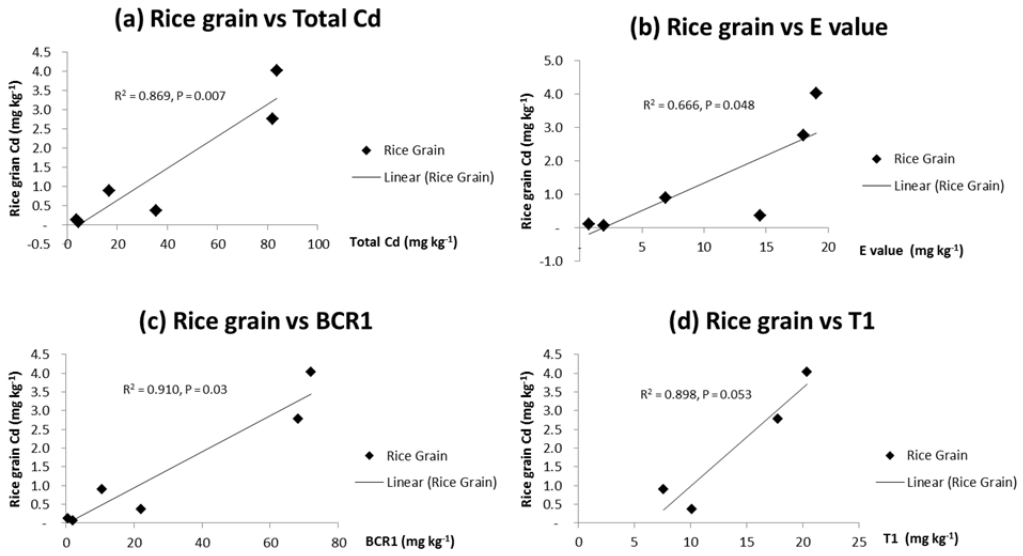


Figure 4.9 Linear regressions between mean rice grain Cd and mean (a) total Cd , (b) E value, (c) BCR1 , (d) T1: exchangeable Cd concentrations in soils

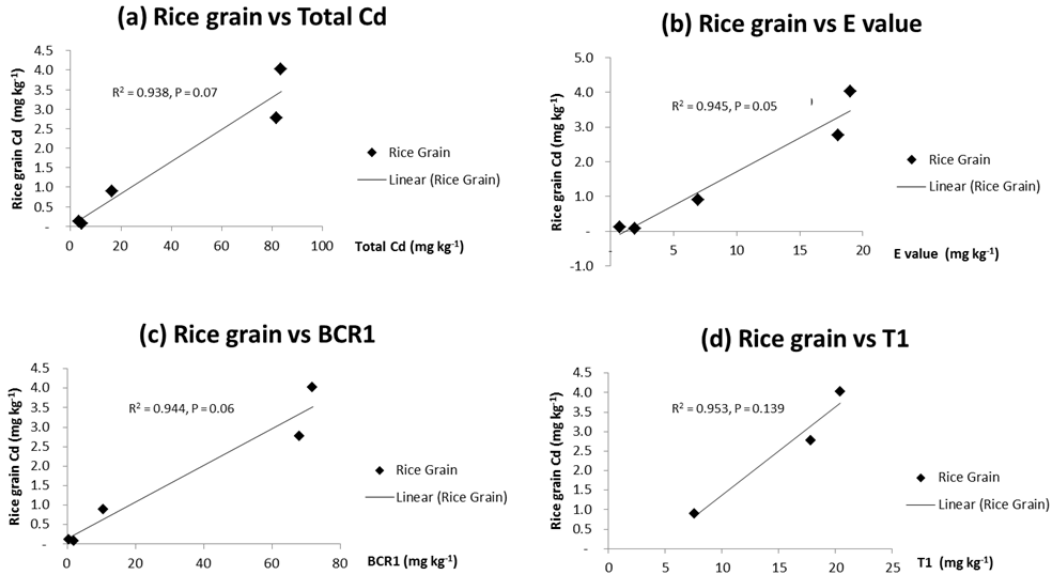


Figure 4.10 Linear regressions between mean rice grain Cd and mean (a) total Cd , (b) E value, (c) BCR1 , (d) T1: exchangeable Cd concentrations in soils (omitting the data for F14)

4.4 Summary and Conclusions

A stable isotope exchange protocol was developed and applied to determine the concentration of labile Cd in selected low-Cd, medium-Cd and high-Cd contaminated paddy soils. ICP-MS, the key instrument used to measure the $^{114}Cd/^{111}Cd$ ratio, displayed high precision and accuracy as evidenced by the linear correlation of the counts and Cd concentration response for the four main naturally occurring Cd isotopes in standard solutions and the stability of the $^{114}Cd/^{111}Cd$ ratios for unspiked soil samples and standard solutions during and between runs. High precision was also displayed in relation to the isotopic ratios determined for the certified reference solution SRM1643.

The E values obtained by this method increased with increasing total Cd content of the soil but there was a major difference between the proportion of labile Cd in the medium- and the high-Cd contaminated soils. The former typically had ~twice the labile Cd compared with the latter.

Comparison between BCR1 Cd and the E values showed that the latter were much lower and this was attributed to the extraction of carbonate-bound Cd in addition to exchangeable Cd during the BCR1 extraction step. The first two steps of the Tessier extraction scheme were then used to distinguish between these two forms of Cd. With the exception of the sample from F14, there was much closer agreement between T1 Cd and the E values.

In a further experiment, T1-exchangeable Cd was first removed and then the E value was determined for the residual soil. On the basis of the close agreement between T1 Cd and the E values for total soil it would have been expected that the E values for the residual would have been near zero. However, the extent of reduction was only ~37-67% of the original E values and so it was clear that the labile (E value) and

exchangeable (T1) pools of Cd were not the same. On the other hand, it may be possible that the Tessier-exchangeable extract altered the surface properties and/or composition of the residual soil such that previously non-isotopically exchangeable Cd was brought into the exchangeable pool.

A conclusion is therefore drawn that while neutral salt extraction may approximate the E value (or isotopically labile pool) for the soils under examination, the isotopically labile pool can have varying contributions from other fractions that are not always directly accessed by the neutral salt extraction and the importance of such contributions may vary among different soils.

In relation to rice grain Cd, when considering all data points, a comparison of total, E values, BCR1 and T1 Cd concentrations with those for Cd in rice grains revealed that BCR1 Cd was the best indicator of the extent of Cd uptake by rice grains (R^2=0.910). This suggests that BCR1 extraction is the most robust of the methods tested for predicting rice Cd concentrations. However, if one soil, F14, was excluded from the dataset, all parameters tested (total, E values, BCR1 and T1 Cd) proved to be effective predictors for rice grain Cd (R^2=0.938-0.953).

In the next chapter, bioavailability assessments are developed further by comparing abiotic measurements (E values and the first-two steps of the Tessier scheme) alongside rice growth experiments.

References

Aggarwal, S.K., N.W. Gemma, M. Kinter, J. Nicholson, J.R. Jr Shipe and D.A. Herold. (1993) Determination of platinum in urine, ultrafiltrate, and whole plasma by isotope dilution gas chromatography-mass spectrometry compared to electrothermal atomic absorption spectrometry. *Anal Biochem* 210(1): 113-8.

Ayoub, A.S., B.A. McGaw, C.A. Shand, and A.J. Midwood. (2003) Phytoavailability of Cd and Zn in soil estimated by stable isotope exchange and chemical extraction. *Plant and Soil* 252: 291–300.

Bermond A. (2001) Limits of sequential extraction procedures re-examined with emphasis on the role of H^+ ion reactivity. *Analytica Chimica Acta* 445: 79-88.

Booth, M.S., J.M. Stark, and E. Rastetter. (2005) Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecological Monographs* 75: 139–157.

Brun, L.A., J. Maillet, P. Hinsinger and M. Pepin. (2001) Evaluation of copper availability to plants in copper-contaminated vineyard soils. *Environmental Pollution* 111(2): 293-302.

de Vries, M.P.C. (1983) Investigations on twenty Australian sewage sludges - their evaluation by means of chemical analysis. *Fertilizer Research* 4: 75-87.

Dicken, A.P. (2005) *Mass spectrometry*. In Radiogenic Isotope Geology. Cambridge. Cambridge University Press. 512 pp.

Echevarria, G., J.L. Morel, J.C. Fardeau and E. Leclerc-Cessac. (1998) Assessment of phytoavailability of nickel in soils. *Journal of Environmental Quality* 27: 1064-1070.

Flynn, K. J. (1998) Estimation of kinetic parameters for the transport of nitrate and ammonium into marine phytoplankton. *Marine Ecology Progress series* 169: 13-28

Fujii, R., and R.B. Corey. (1986) Estimation of isotopically exchangeable cadmium and zinc in soils. *Soil Science Society of America Journal* 50: 306-308.

Huang, Z.Y., T. Chen, J. Yu, X.C. Zeng and Y.F. Huang. (2011) Labile Cd and Pb in vegetable-growing soils estimated with isotope dilution and chemical extractants. *Geoderma* 160(3-4): 400-407.

Hutchison, J.J., S.D. Young, S.P. McGrath, H.M West, C.R. Black and A.J.M. Bakers. (2000) Determining uptake of `non-labile' soil cadmium by *Thlaspi caerulescens* using isotopic dilution techniques. *New Phytol*. 146: 453-460.

Kirkham, M.B. (2006) Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation and amendments. *Geoderma* 137(1-2): 19-32.

Li, Z., R.G. McLaren and A.K. Metherell. (2004) The availability of native and applied soil cobalt to ryegrass in relation to soil cobalt and manganese status and other soil properties. *New Zealand Journal of Agricultural Research* 47: 33-43.

Mann, G.E., J.D. Pearson, C.J. Sheriff and V.J. Toothill. (1989) Expression of amino acid transport systems in cultured human umbilical vein endothelial cells. *Journal of Physiology* 410: 325-339

McGrath, S.P. and J. Cegarra. (1992) Chemical extractability of heavy metals during and after long-term applications of sewage sludge to soil. *Journal of Soil Science* 43: 313-321.

Nakhone, L.N. and S.D. Young. (1993) The significance of (radio-) labile cadmium pools in soil. *Environmental Pollution* 82(1): 73-77.

Nolan, A.L., Y. Ma, E. Lombi and M.J. McLaughlin. (2004) Measurement of labile Cu in soil using stable isotope dilution and isotope ratio analysis by ICP-MS. *Analytical and Bioanalytical Chemistry* 380(5-6): 789–797.

Oliver, I.W., Y. Ma, E. Lombi, A.L. Nolan and M.J. McLaughlin. (2006) Stable Isotope Techniques for Assessing Labile Cu in Soils: Development of an L-Value Procedure, Its Application, and Reconciliation with E Values. *Environ. Sci. Technol*. 40(10): 3342-3348

Simmons, R. W., A. D. Noble, P. Pongsakul, O. Sukreeyapongse and N. Chinabut. (2008) Analysis of field-moist Cd contaminated paddy soils during rice grain fill allows reliable prediction of grain Cd levels. *Plant and Soil* 302(1-2):125-137

Smolders, E., K. Brans, A. Foldi and R. Merckx. (1999) Cadmium fixation in soils measured by isotopic dilution. *Soil Science Society of America Journal* 63: 78-85.

Hamon, R., J. Wundke, M. McLaughlin and R. Naidu. (1997) Availability of zinc and cadmium to different plant species. *Australian Journal of Soil Research* 35(6): 1267-1277.

Hutchinson, J.J., S.D. Young, S.P. McGrath, H.M. West, C.R. Black and A.J. Baker. (2000) Determining uptake of 'non-labile' soils cadmium by *Thlaspi caerulecens* using isotopic dilution techniques. *New Phytol* 146: 453-460.

McLaren, R.G. and D.V. Crawford. (1974) Studies on soil copper. III. Isotopically exchangeable copper in soils. *Journal of Soil Science* 25(1): 111-119.

Sterckeman, T, J. Carignan, I. Srayeddin, D. Baize, C. Cloquet. 2009. Availability of soil cadmium using stable and radioactive isotope dilution. *Geoderma* 153: 372–378.

Tessier, A., P.G.C. Campbell and M. Bisson. (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* 51: 844-851.

Tiller, K.G., J.L. Honeysett and M.P.C. de Vries. (1972) Soil zinc and its uptake by plants. I. Isotopic exchange equilibria and the application of tracer techniques. *Australian Journal of Soil Research* 10(2) : 151-164.

Ahnstrom, Z.A., and D.R. Parker. (2001) Cadmium reactivity in metal-contaminated soils using a coupled stable isotope dilution-sequential extraction procedure. *Environ Sci Technol.* 35(1):121-126.

Tongtavee, N., J. Shiowatana., R.G. McLaren and C.W. Gray. (2005) Assessment of lead availability in contaminated soil using isotope dilution techniques. *Science of the total environment* 348(1-3): 244-256.

Wendling, L.A., J.K. Kirby and M.J. McLaughlin. (2008) A novel technique to determine cobalt exchangeability in soils using isotope dilution. *Environmental Science & Technology* 42(1): 140-146.Sinaj, S., A. Dubois and E. Frossard. (2004) Soil isotopically exchangeable zinc: A comparison between E and L values. *Plant and Soil* 261: 17-28.Lombi, E., R.E. Hamon, S.P. McGrath and M.J. McLaughlin. (2003) Lability of Cd, Cu, and Zn in

polluted soils treated with lime, beringite, and red mud and identification of a non-labile colloidal fraction of metals using isotopic techniques. *Environmental Science and Technology* 37(5): 979-984.

Xie, H, Z-Y. Huang, Y-L. Cao, C. Cai, X-C. Zeng and J. Li. (2012) Labile pools of Pb in vegetable-growing soils investigated by an isotope dilution method and its influence on soil pH. *Journal of Environmental Monitoring* 14(8): 2230-2237.

Young, S.D., A. Tye, A. Carstensen, L. Resende and N. Crout. (2000) Methods for determining labile cadmium and zinc in soil. *European Journal of Soil Science* 51(1): 129-136.

Young, S.D., H. Zhang, A.M. Tye, A. Maxted, C. Thums and I. Thornton. (2006) Characterizing the availability of metals in contaminated soils. I. The solid phase: sequential extraction and isotopic dilution. *Soil Use and Management* 21(s2): 450-458.

Chapter 5 Utility of Biochars (Chars) for Restricting Cd Bioavailability in Medium and High Cd-contaminated Paddy Field Soils from Mae Tao Watershed, Northwestern Thailand

5.1 Introduction

In previous chapters the bioavailability of Cd in the soils of Mae Tao watershed, northwestern Thailand was examined abiotically, through the employment of sequential extractions (Tessier and BCR schemes) and isotopic exchange techniques (E values). Substantial proportions of the soil Cd were found to be labile and thus potentially bioavailable, i.e. 28-45% in medium Cd-contaminated soils (see Chapters 3 and 4). High Cd-contaminated soils also had potentially available proportions approximating 20-22% of total Cd which, although being a lower percentage relative to the values in medium Cd-contaminated soils, still represents a sizeable amount of Cd considering that the total Cd concentrations in the highly contaminated fields were of the order of 80 mg kg^{-1}. The high amounts of potentially available Cd helped to explain the high rice Cd concentrations observed in this study (Chapter 3) and previously recorded in Mae Sot district (Chapters 1 and 2). Moreover, it emphasizes the need to address the issue of Cd uptake by rice and the associated human health consequences already discussed (Chapters 1 and 2; also Kosolsaksakul *et al.,* 2014). Therefore in this Chapter the uptake of Cd into rice is examined along with a potential mitigation strategy: the use of biochar to reduce Cd bioavailability to rice plants.

5.1.1 Biochar and Its Application to Soils

As succinctly stated by Lehmann and Joseph (2009), "biochar is the carbon-rich product obtained when biomass, such as wood, manure or leaves, is heated in a

closed container with little or no available air". More specifically, biochar is the material created via the thermal decomposition, or pyrolysis, of organic material under limited supply of oxygen at relatively low temperatures (generally <700°C; Lehmann and Joseph, 2009). Production of biochar and its subsequent incorporation into soil has been widely hailed as a potential climate change mitigation strategy, i.e. growth of plant material captures CO_2 from the atmosphere and the conversion of this biomass into biochar produces a stable form of C that can then be incorporated into soil for long term storage (the stability of biochar in soil is currently estimated at hundreds to thousands of years) (Lehmann and Joseph 2009; Sohi *et al.,* 2009; Downie *et al*., 2011).

Biochar incorporation has also been shown to have soil management and agricultural benefits, chiefly by increasing the recalcitrant organic matter content of soil and thereby decreasing bulk density and increasing the porosity, water holding capacity and cation exchange capacity (see reviews by Beesley *et al*., 2011; Huang *et al.*, 2013; McHenry 2009; Sohi *et al.*, 2009). The most notable (and most often cited) example of this beneficial effect is in relation to the *Terra Preta de Indio* soils of Amazonia, being anthropogenic dark earths thought to date back several thousand years and which have elevated fertility and yield compared with the low fertility soils that surround them (Lehmann and Joseph 2009; Sohi *et al.,* 2009). However, in addition to these beneficial effects, recent studies have demonstrated biochar's potential capacity for sorbing contaminants in soils and thereby reducing contaminant mobility and possible bioavailability. Promising results have been shown for organic contaminants (Kookana *et al.*, 2010; Martin *et al.,* 2012), excess nutrients (Zheng *et al.*, 2013; Xu *et al*., 2014) and metals (Beesley and Marmiroli 2011; Houben *et al.,* 2013; Fellet *et al.*, 2014).

The structure of biochar, being porous with high surface area and with reactive surfaces, is what leads to this sorption capacity (Lehmann and Joseph 2009; Sohi *et al*., 2009). Recent work has focused on identifying the surface functional groups on

biochar, with one study concluding that biochar in terra preta soils consists predominantly of char residues composed of ~6 fused aromatic rings that are surface-substituted by COO^- groups and, moreover, that it is these surface groups that significantly increase a soil's cation-exchange capacity and thus its retention capacity for contaminants and nutrients (Mao *et al.,* 2012). Related structures and functional groups in biochars produced commercially and/or in research laboratories may similarly be responsible for high sorbing capacity.

While biochar can be produced from a great variety of feedstock (e.g. wood, grain residues, straw, nutshells, seeds/pips, poultry and cattle manure, paper pulp and sewage sludge), only locally available and renewable materials can be considered as a truly viable option for rural areas in developing countries, such as the Mae Sot region of Thailand examined in this study. For this reason, biochars produced from rice husk and from miscanthus (a perennial grass that commonly occurs across much of Asia) were considered in this research because both feedstock materials are abundantly available in this agricultural region of Thailand. For example, rice husk, the brown hard outer hull of rice grain, is the residual from the rice winnowing process and it is plentiful and cheap in Thailand. Indeed, many farmers simply leave it at the rice mill to be disposed of, while others use it as a soil improvement amendment (i.e. for increasing organic matter content) or convert it into chars that can be used as a medium for geminating young plants (Boonruang Tawong, pers. comm., 2010).

The aims of this chapter were: (1) to study the properties of two chars (produced from rice husk and miscanthus, at the University of Edinburgh), with especial focus on their potential Cd sorption capacity; (2) to assess the capability of rice husk char (RHC) in reducing the labile Cd (E value) in the medium and high-Cd contaminated paddy field soils; (3) to evaluate the effectiveness of RHC in reducing uptake of Cd by rice plants grown in char-amended medium- and high-Cd contaminated soils; and

(4) to compare the associations of Cd in char-amended medium- and high Cd-contaminated soils before and after the rice plant uptake experiments.

5.2 Experimental Methods

5.2.1 Initial Biochar Production and Physical Property Assessment

Two rice husk chars (RHC) (Figure 5.1) and two miscanthus chars (MC) were produced by pyrolysis at each of two distinct temperatures: 350ºC and 700ºC (provided by the Biochar Research Centre, University of Edinburgh).

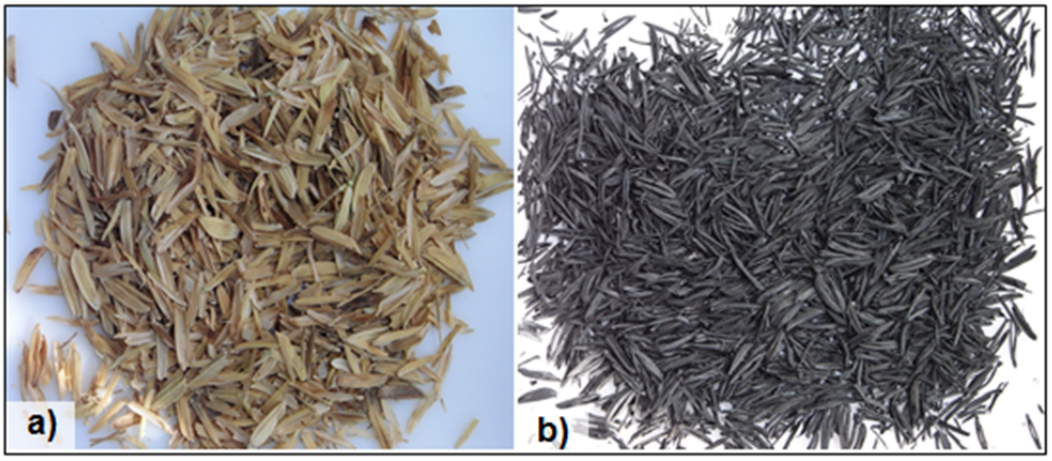


Figure 5.1 Photographs of the (a) whole grain rice husks and (b) whole grain rice husk char (700ºC pyrolysis)

5.2.1.1 FTIR Characterisation of Surface Functional Groups

In order to determine which of the chars would be most suitable for the bioavailability and rice growth study, Fourier Transform Infra-red (FTIR) was used

to assess surface properties, specifically the functional groups to which metal ions might bind. For FTIR analysis, potassium bromide (KBr) discs were prepared by mixing 1.5 mg dried and ground biochar samples with 200 mg dried IR grade KBr using an agate pestle and mortar. The homogeneous powder was then placed between stainless steel discs, pressure (~10 tonnes for ~1 minute) was applied by hydraulic press (Specac) and then translucent disc was transferred into the disc holder of the FTIR spectrometer (Perkin Elmer spectrum 65) for analysis. The details of analysis conditions were as follows. The scanned wavenumber range was 4000-400 cm^{-1}; the scanning increment was 2 cm^{-1}; the number of scans was 12 and background correction was achieved using a blank KBr disc (no sample).

5.2.1.2 SEM Characterisation of Biochar Structure

Physical structure analysis was conducted by SEM. The samples were prepared by grinding the biochar to a fine powder which was transferred onto a metal stub and was then gold-coated before analysis as this produces clearer images (EPA, 2002). Analysis was carried out on a Philips XL30CP with PGT Spirit X-ray analysis and HKL Channel5 Electron Backscatter Diffraction (EBSD) systems.

5.2.1.3 Determination of Cation Exchange Capacity of Biochar

Cation exchange capacity (CEC) analysis followed the method of Sumner and Miller (1996) 1.5 g of char (n=3) was placed in a 50 ml centrifuge tube to which was added 33 ml of a 0.2 M $CaCl_2$ - 0.0125 M $CaSO_4$ solution. The tubes were then shaken for 15 minutes, centrifuged for 15 minutes at 7000 rpm (6793 g) and the supernatant discarded. The addition of the Ca solution, shaking and centrifugation steps were repeated twice (i.e. 3 times in total) to ensure Ca saturation of exchange sites. Then the samples were washed to remove excess Ca ions with deionised water many times

until very low Ca concentrations (<1 mg L^{-1}; determined by ICP-OES as described below) were found in the wash solutions.

After that, 33 ml 0.2 M $Mg(NO_3)_2$ was added to displace the Ca ions from the exchange sites and the tubes were shaken for 15 minutes, centrifuged for 15 minutes (7000 rpm, 6793 *g*) and then the supernatant was filtered via a Whatman 42 filter paper into a 100 ml volumetric flask. This was repeated a further two times and the combined supernatants were finally made to volume (100 ml) using deionised water. To determine the displaced Ca (and thus allow determination of CEC), all solution samples were analysed for Ca concentration by ICP-OES (see Chapter 3, section 3.1.3.2, for ICP-OES analysis settings and procedures).

5.2.2 Abiotic Assessment (E values) of the Feasibility of Using Biochar to Reduce Cd Bioavailability in Paddy Soils

The medium-Cd soil (F14; total Cd 61.5 mg kg^{-1}*) and high-Cd (F10; total Cd 83.7 mg kg^{-1}**) contaminated soils, from the same fields assessed in Chapter 4 for available Cd using the stable isotope exchange (ISE, E-value) method, were tested here to determine the mitigating effect that biochar addition could have on available Cd. On account of the FTIR and CEC measurements (see results section 5.3.1), the RHC produced at 350ºC and at 700ºC as well as the MC produced at 350ºC were separately tested for their effects on Cd E-values. The chars were added to soils in proportions of 0, 1, 5, 10, 15 and 20% w/w. Before mixing, the chars were prepared by grinding into a fine powder using a pestle and mortar. The required amount of

* These soils were collected in a small ziplock bag during a first trip to Thailand where the purpose was to collect soil for the biochar experiments. Although collected from the same field, the concentration of Cd was greater than that determined in the initial soil samples collected from F14 (a composite sample - 35.4±4.34 mg kg^{-1}) and exceeded the medium-Cd soils zoning (20-50 mg kg^{-1}) used in earlier chapters. However, as it was still lower than the concentrations in F10 (83.7 mg kg^{-1}; composite sample in the 1st collection and 71.2 mg kg^{-1} in the 2nd collection for the rice growing experiment), the high-Cd contaminated soil, the designation of 'medium-Cd' was retained.

** This soil was a composite sample collected during the first trip to Thailand.

char was then weighed into a 50 ml centrifuge tube, followed by a weighed amount of soil, e.g. for the 1% w/w experiment 0.1 g char and 9.9 g of soil was added to the tube. Then 20 ml 0.01 M $MgCl_2$ was added and the resulting slurries were mixed by shaking for two days before starting the isotope spiking process. The stable isotope exchange (SIE) technique was then conducted as described in Chapter 4. Samples of RHC and MC were also subjected to the SIE method so that E values for the chars themselves could be evaluated.

5.2.3 Biotic Assessment (Rice Plants) of the Utility of Biochar for Restricting Cd Bioavailability in Paddy Soils and Comparison with E values and Tessier Extractions

Since the results of the abiotic assessment described above (see results section 5.3.2) revealed a reduction in E values for medium Cd-contaminated soil amended with RHC, a rice growth study was embarked upon to further test the capacity of this biochar to reduce the bioavailability of Cd and thus ultimately limit its assimilation into rice.

5.2.3.1 Soils, Rice Seeds and Rice Husks

The soils, rice seeds and rice husks for the rice growing experiment were imported from Thailand in October 2012. The soils were from the medium Cd-contaminated fields (F4 and F14) and high Cd-contaminated fields (F10 and F18). A large amount of topsoil (0-20 cm) was collected in the middle of each field using a clean spade. The soils (90 kg in total) were air-dried before transporting to the UK. Rice seeds were of the variety Khao Dawk Mali 105 (called Jasmine variety in Thailand). This variety was selected because it is the type grown by the farmers in the study fields in Mae Sot. Rice husks were also imported from Thailand in order to produce RHC at the UK Biochar Research Centre at the University of Edinburgh. The RHC for the

rice growth experiments was produced by pyrolysis at 350ºC and was ground to a fine powder for use in soil treatments.

5.2.3.2 Soil and Treatment Preparation

A medium-Cd contaminated soil was prepared for use in the rice growth experiment by mixing two of the medium-Cd soils together, soils F4 (total Cd 13.4±0.08 mg kg^{-1}) and F14 (total Cd 26.5±0.72 mg kg^{-1})*. Approximately 25 kg of each of the soils were mixed and homogenized by hand in a large plastic bag for 15-20 minutes. Similarly, a high-Cd contaminated soil was prepared for use in the rice growth experiment by mixing two of the high-Cd soils together, soil F10 (total Cd 71.2±2.11 mg kg^{-1})* and F18 (66.2±2.02 mg kg^{-1}). The total Cd concentrations in the mixed soils were determined by ICP-OES following microwave assisted HF/HNO_3 digestion as described in Chapter 3.

Treatments were established as control (0% char), soils with 1% RHC w/w amendment and 5% RHC w/w amendment for each of the prepared medium-Cd and high-Cd contaminated soils (Table 5.1). The total soil or soil+biochar weight in each pot was 1 kg. Fertiliser, used in Thailand (1.8 g of 18-6-6, N-P-K from Thailand) was added into each pot and mixed with the soils before growing rice.

* These soils were collected during the second trip to Thailand where the purpose was to collect soil for the rice growing experiment.

Table 5.1 Treatments for Rice Growing Experiment

	Control (0% chars)	**1% RHC (350°C)**	**5% RHC (350°C)**	**total**
medium-Cd contaminated soils	6 pots (4)*	3 pots (3)	3 pots (3)	12 pots (10)
high-Cd contaminated soils	6 pots (4)	3 pots (2)	3 pots (2)	12 pots (8)
Total	12 pots (8)	6 pots (5)	6 pots (5)	24 pots** (18)

* (surviving pots)

** The capacity of the growth tent (i.e. the amount of space inside) limited the growth experiment to 24 pots. The mass of available soil from Thailand also restricted the scale of the growth experiments.

5.3.3.3 Growth Experiment Equipment and Achievement of Optimal Rice Growing Conditions

Rice was grown in a temperature controlled 'growth tent', the details of which were:

(1) Dimensions of 80 cm wide by 80 cm depth and 1.8 m height, with the inside coated in silver reflective fabric and a closable door (tent zipper);

(2) Light bulbs (Enviro Gro Cool White CFL 250W) suspended from a bar attached to the roof of the tent;

(3) Temperature controlled heaters (Slimline Eco-heater 55W);

(4) Thermometer (including max-min readout);

(5) Humidifier (Mystery-Wonder Mini-Fogger 240V)

For the reasons given in more detail below, the aim was to achieve the optimum conditions for rice growing which were high humidity (>60%), high temperature

(25-30°C) and light at 120-150 µmol $m^{-2}s^{-1}$ (8,880-11,100 Lux-Fluorescent) as recommended by Kaewchumnong *et al.* (2008).

As described in Chapter 2.1.1, the growing season in Thailand normally runs from July to November every year, which coincides with the rainy season during which high humidity (over 60%) conditions and temperatures of about 25-30ºC prevail. The growing tent therefore was set up to simulate as far as possible the Thai conditions. Kaewchumnong *et al.* (2008) recommended the use of fluorescent light at 120-150 µmol $m^{-2}s^{-1}$ (8,880-11,100 Lux-Fluorescent) and this was achieved with two plant growth fluorescent bulbs (250 W), with a third added once the rice plants had reached 90 days (Figure 5.2). The light was provided 12 hours per day (about 6 am – 6 pm), in line with the usual hours of daylight in Thailand.

The temperature was raised to 25ºC by heaters. Humidity was set at 60-70% and was controlled with the humidifiers and the placement of two buckets of water inside the tent. Later in the experiment a third water bucket was added to maintain constant humidity. Two small fans were also set up to circulate the air and moisture throughout the tent (Figure 5.2).

Temperature and humidity were monitored and when necessary (i.e. during summer) the tent zipper was partly opened and the light bulb position (height above plants) was adjusted. A clear plastic sheet was added in between the bulbs and the rice plants in order to maintain the high humidity (Figure 5.2). Throughout the growth experiment period, the temperature in the growth tent was 22-27ºC, light intensity was 10,000-14,000 lux and the humidity was 50-80% (mostly 65-70%).

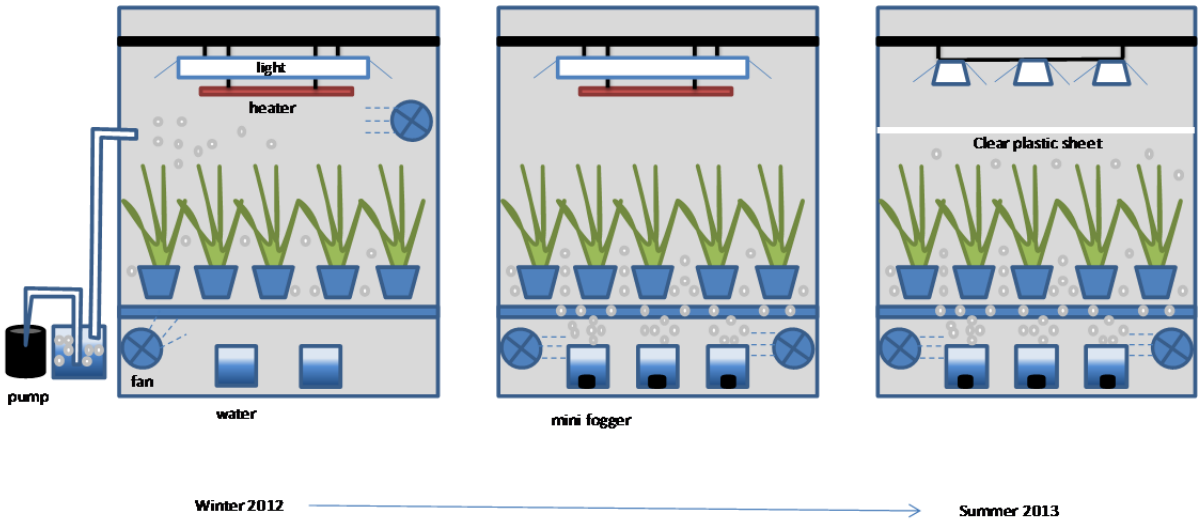


Figure 5.2 The growth tent and the adjustment of accessories during the experiment

5.2.3.4 Germination and Rice Growing

The current rice growing best practice followed by farmers in Mae Sot involves soaking rice seeds in water for 1-2 nights and then placing them on a plastic tray which had pores at the bottom and which had been lined with whole grain (i.e. not ground to a fine powder) rice husk char (Figure 5.3). A very thin layer of whole grain rice husks is then placed on top and the germinating seeds are watered and the moisture content kept high for 3-4 days. After this initial wetting period, the trays are moved to a germination/establishment field for about 18 days before the young plants are finally removed from the tray and transplanted to the rice fields. (Boonruang Tawong, pers. comm., 2010; see also www.suansing.com, http://www.youtube.com/watch?v=ulTk10WE2pY).

Figure 5.3 The current rice germination technique where rice seed is placed in a plastic tray lined with whole grain rice husk chars (http://www.suansing.com)

Growing rice in Edinburgh presented a challenge and required the development of a modified approach, but one that still reflected rice growing practices at the study site. Development of a viable methodology for germinating rice seeds, transplanting them to test soils and getting them to grow into established plants in the Edinburgh laboratory took approximately six months of trial and error. Table 5.2 summarises, and Appendix 5.1 describes in detail, the trial and error process that led to the optimal method described below.

Table 5.2 Summary of Germination and Rice Growing Trials

Method	Germination medium	Result	Transplantation to test soil	Result
1	clay soils	Just developed; roots not developed well; shallow roots	Not transplanted	-
2	sand + clay + whole grain RHC (1:1:1 mixture)	Good development	Transplanted at 2-3 weeks – roots with no soil attached	Poor growth; died at one month old
3	Sand + clay + whole grain RHC (1:1:1 mixture)	Good development	Transplanted at 2-3 weeks – roots with soil attached	Poor growth; died at one month old
4	whole grain RHC	Poor development	Transplanted at 2-3 weeks – roots with no soil attached	Poor growth; not viable
5	Clay with a 1-2 cm layer of whole grain RHC on top; carried out in geminating tray (Figure 5.4)	Good development; roots penetrated to clay layer	Transplanted at 2-3 weeks – roots with no soil attached	Good initial growth but died at one month old
6	Clay with a 1-2 cm layer of whole grain RHC on top; carried out in a polystyrene cup with the base removed, placed at the centre of pots	Some good development but some had roots that did not penetrate to clay layer	-	Some good growth, but numerous plants died at about two months old
7	Clay with 1-2 cm layer of whole grain RHC on top; in pots	Good germination and shoot development; roots also developed very well and penetrated deep into clay layer	Transplanted at 1-2 months with soil attached (Figure 5.5)	Most plants grew well, a few died at 1-2 months old (Figure 5.6 and 5.7)

Figure 5.4 The rice seeds germinating in the whole grain RHC on top of the clay soil (see Table 5.2 Method 5)

Figure 5.5 The rice plants after transplanting (one and a half months old) (see Table 5.2 Method 7)

Figure 5.6 The rice plants aged about 3 months (see Table 5.2 Method 7)

Figure 5.7 The rice plants before harvesting (nearly four months old) (see Table 5.2 Method 7)

In the optimal method, rice seeds were germinated by soaking in deionised water for 1-2 nights and then placing on wet paper towelling. Then the young rice was grown in the germinating pots containing clay soil with 1-2 cm whole grain RHC (Table 5.2; method 7). The rice plants grew well (~12-15 cm above ground height) and, after 1-2 months, they were then moved to the growing pots. By adding deionised water, the water level was always kept about ~2-3 cm above the soil surface except during fertiliser addition. About one month after transplanting, the water level was allowed to drop and 1.8 g fertiliser (N-P-K: 18-6-6) was added to each pot (the same rate used in the fields) before re-submerging the soils again. The growth tent conditions were controlled throughout the growing period (see details in section 5.2.3.3). After almost four months (restricted by remaining time for the experiment), the harvest started by draining the water out of the soils by removing the surface water above the soil using a syringe and letting them dry for 3-4 days. The whole rice plants were carefully pulled out with their roots intact. The timeline of rice growing is shown in Table 5.3 below.

Table 5.3 Timeline of the Rice Growing Experiment (The Definitive Method)

Activities	Date
Germination	26 April 2013
Transplantation	15 June 2013
Addition of fertiliser	15 July 2013
Harvest	13 August 2013

In preparation for analysis, each rice plant was first washed with tap water to remove excess soil and then rewashed with deionised water many times until all soil particles were removed from all parts of the plants, especially the root areas. After that the rice plants were dried in the oven at 65°C for three days (until constant weight was reached). Then the whole rice plants were weighed and divided into root and straw parts. The weights of each part were also recorded. The root and straw samples were each cut into small pieces before ball-milling to a fine powder. Finally all powdered

plant samples were digested by USEPA 3051a method and analysed by ICP-OES using the same procedures as in Chapter 3.1.3.3.

5.2.3.5 Total Cd, E values and Tessier Extractable Cd Content of Soils Used in the Plant Growth Study

The Cd contaminated soils used for the rice plant growth experiments (before and after) were analysed for total Cd concentration, E value and Cd concentrations in the first two Tessier sequential extraction steps. In brief, total Cd concentrations were determined following microwave-assisted acid digestion using 15.8 M HNO_3 (Aristar)/22.6 M HF (Aristar) (as described fully in Chapter 3). E values were determined after spiking the soil with a stable isotope (^{111}Cd) using a solution of 0.01 M $MgCl_2$ (as per Chapter 4). The first step of the Tessier scheme (i.e. 1 M $MgCl_2$ at pH 7, targeting the exchangeable fraction), with the residues then subjected to the second step (i.e. 1 M CH_3COOH at pH 4.5, targeting the carbonate bound fraction), was conducted as described in Chapter 3. All solutions were analysed for Cd by ICP-OES. Analysis of variance (ANOVA) and the least significant difference (LSD) analysis were used to analyse the relationship between the Cd concentrations in the soil extracts and the Cd concentrations in the rice plants. The level of significance was $p<0.05$.

5.3 Results and Discussion

5.3.1 Initial Biochar Production and Physical Property Assessment

5.3.1.1 FTIR Analysis of Biochars

Figure 5.8 shows the FTIR spectra for the RHC produced at both 350°C and 700°C and the MC produced at 350°C.

All three chars had similar functional groups which are defined below.

- Non-H bonded OH groups: sharp peaks at 3500, 3435, 3390 cm^{-1} (MC 350°C) and 3550, 3478, 3413, 3272 cm^{-1} (RHC 700°C)
- H-bonded OH groups: broad band at 3692-2996 cm^{-1} (MC 350°C)
- Aryl C-H (stretch): tiny sharp peaks at 3062 cm^{-1} (RHC 350°C) Alkyl C-H (stretch): sharp peaks at 2926-23, 2926, 2869 cm^{-1} (MC 350°C) and 2972, 2924, 2868-2866 cm^{-1} (RHC 350°C)
- Aryl C=C: two sharp peaks at 1638 cm^{-1} and 1618 cm^{-1} (RHC 700°C)
- Carbonyl C=O: broader peak at 1697, 1378 and 1227 cm^{-1} (RHC 350°C) and 1680, 1432, 1226 and 1218 cm^{-1} (MC 350°C)
- Alcohol/phenol O-H bend: sharp peaks at 1378 cm^{-1} (RHC 350°C) 1386-1367 cm^{-1} (RHC 700°C) and 1380 cm^{-1} (MC 350°C)
- Alcohol C-O : broad peak at 1106 cm^{-1} (RHC 350°C), 1095 (RHC 700°C) and 1108 cm^{-1} (MC 350°C)
- Aryl C-H (bend): peaks at 873, 806, 686 and 617 cm^{-1} (RHC 350°C), 874, 806, 799 and 620-621 cm^{-1} (RHC 700°C) and 874-872, 812, 756-754 and 646-642 cm^{-1} (MC 350°C)

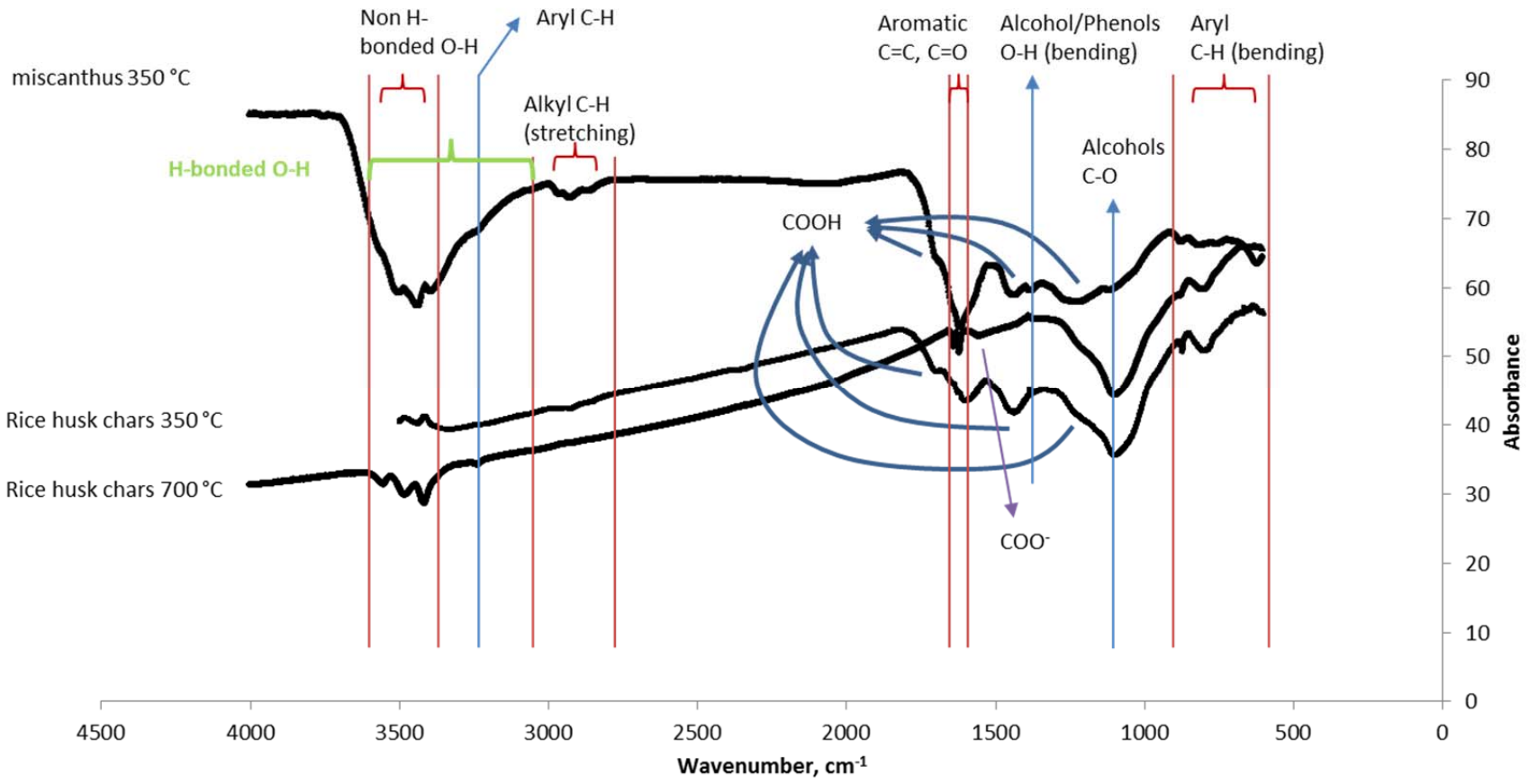


Figure 5.8 The FTIR transmittance spectra for RHC (350°C and 700°C) and MC (350°C)

The general observations about The FTIR spectra for these three biochar materials were as follows. The 1400-1700 cm^{-1} region of the spectrum for RHC produced at 700°C was relatively featureless in comparison with those for the chars produced at 350°C. In particular, it was clear that the two biochars produced at 350ºC had more carboxyl and carbonyl functional groups than that processed at 700ºC. Instead, two sharp peaks at 1638 and 1618 cm^{-1} indicated the presence of aryl C=C groups, consistent with a highly aromatic, poorly oxygenated structure. Thus there were clear effects of temperature on the carboxyl/carbonyl functionality of the biochar product. In contrast, however, there were some spectral features that were observed only for the chars produced from rice husk, i.e. these were temperature-independent features. For example, the alcohol C-O band at 1106, 1095 and 1108 cm^{-1} (RHC 350°C, RHC 700°C and MC 350°C).

These results matched well with Uchimiya's work* (Uchimiya *et al.*, 2013). They studied the influence of temperature on biochars' (cottonseed hulls) functional groups and observed (i) decreasing % transmittance for carboxyl and carbonyl functional groups and O-H stretching when the temperature was greater than 350°C; and (ii) alkyl C-H (stretching) bands only when the pyrolysis temperature was in the range 200-350°C. However, they found that aromatic (C=C) stretching bands also decreased at higher temperature (over 500°C) but these bands were still detected in this study when the pyrolysis temperature was 700°C. Another study (Mimmo *et al.*, 2014), testing the effect of pyrolysis temperature on MC properties, also confirmed the disappearance of carbonyl and hydroxyl groups at pyrolysis temperatures greater than 360°C. Thus, it was considered that the low temperature chars were more suited to adsorbing Cd from the contaminated paddy soils because of their greater carboxyl/carbonyl functionality.

* The cottonseed hulls were pyrolysed at 200, 350, 500, 650 and 800°C for 4 hours under 1,600 mL min^{-1} N_2 flow rate using a laboratory scale box furnace (22 L void volume) with a retort (Lindberg, Type 51662-HR, Watertown, WI) (Uchimiya *et al.*, 2013)

5.3.1.2 Physical Structural Characterisation of Biochars using SEM

In this study, SEM analysis was conducted only for RHC (700°C) because of time limitations. It is included here, however, to show the typical porous nature of biochar. Figure 5.9(a) shows the structure of a small particle of RHC. It had a tubular structure and dimensions ~150 µm length and 70-80 µm diameters. There were many cracks and pores both across and along the particle. Figure 5.9(b) showed clearly that the large pores through the particle were bigger, sized ~20 µm, while the very tiny pores on the surfaces of the particle were only sized ~2-3 µm.

These porous structures are indicative of high internal surface areas. This combined with the prevalence of acidic functional groups (see a previous section) potentially gives the chars a high capacity to adsorb water and heavy metals. Cation exchange capacity as determined in the next section is a means of quantifying the acidic sites with which metals can interact. The complexed heavy metal can exchange with functional groups present in the biochar, and the cations associated with the biochar such as Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and S as well as physical adsorption (Paz-Ferreiro *et al.*, 2014). Moreover the sorption of the metal e.g. Cd, As and Zn which was produced at the biochar surface was not immediately reversible (Beesley and Marmiroli, 2011).

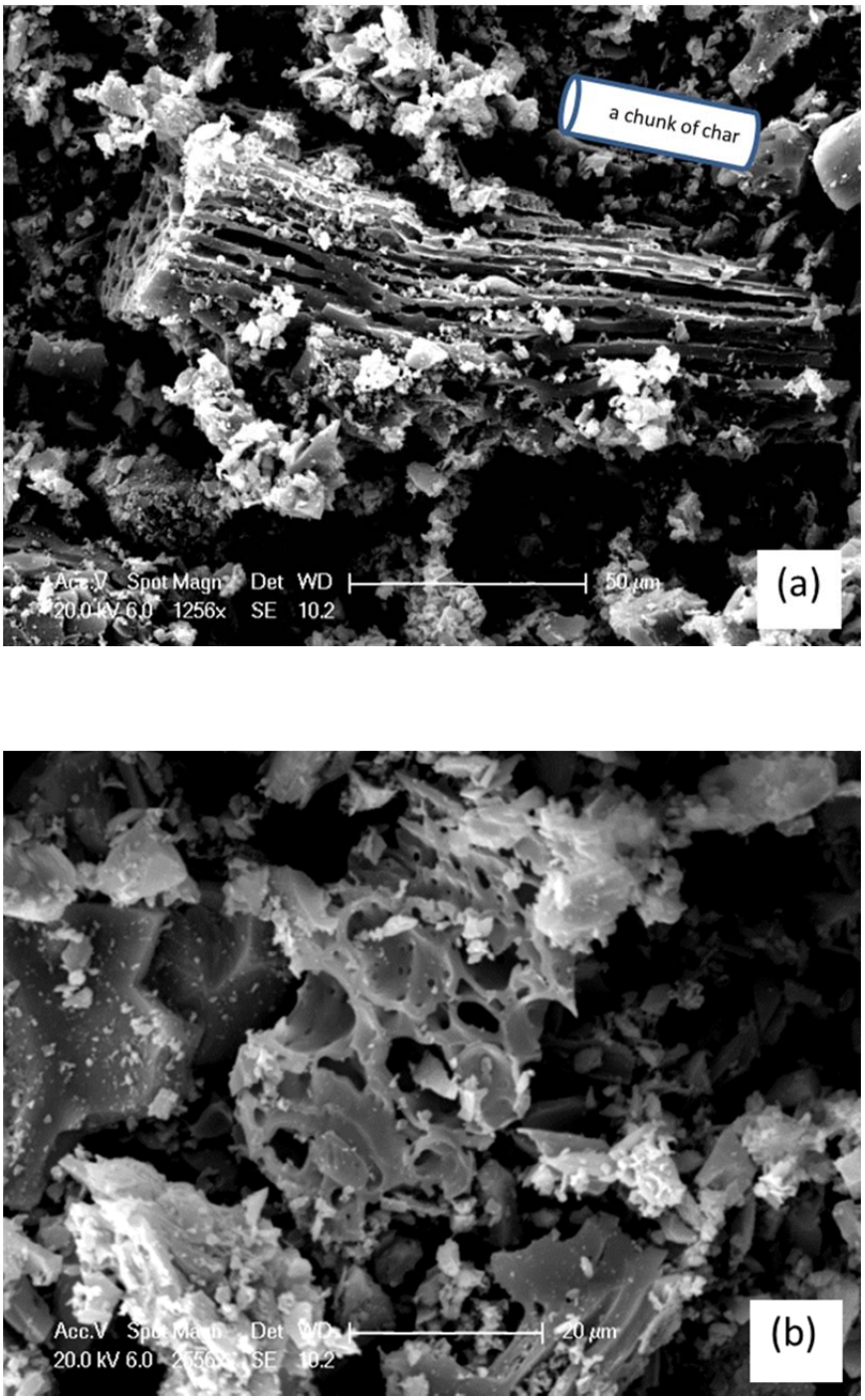


Figure 5.9 SEM image showing the structure of rice husk chars (700ºC pyrolysis) (a) image of a RHC particle showing a tubular structure with pores across and along the particle (scale shown on image = 50 µm) and (b) Enlarged SEM image of the RHC particle showing the tiny pores along the surface of RHC (scale shown on image = 20 µm)

5.3.1.3 Cation Exchange Capacity (CEC)

In this study CEC was used as a tool to investigate the capacity of biochars to exchange and therefore possibly retain cations, including heavy metals such as Cd.

The results showed that the CEC of both RHC and MC pyrolysed at 350ºC were higher than the chars pyrolysed at 700ºC (Table 5.4). This is in agreement with Chen *et al*. (2011), who tested the properties of orange pip chars (ORC) produced at different temperatures (250ºC, 400ºC and 700ºC) and found that at 250ºC functional groups, e.g. aliphatic CH, ester C=O, aryl C=O and C=C, aryl CO and aryl OH, were more preserved than at higher temperature pyrolysis. However ORC produced at 250ºC and 400ºC had lower total pore volumes (0.0592 and 0.0409 cm^3 g^{-1}, respectively) than that produced at 700ºC (0.390 cm^3 g^{-1}). Thus, pyrolysis at high temperature (e.g. 700°C) produces chars of greater internal surface areas than pyrolysis at lower temperature (250-400°C). Cation exchange capacity is obtained by multiplying the concentration of ion exchange sites by surface area. It would appear that functionality rather than surface area is the most important factor contributing to the CEC of the biochar samples. Since both the RHC and MC produced at 350°C had higher CEC values than the 700°C char, the former were used in the abiotic assessment of biochar as a means of reducing Cd bioavailability in paddy field soils. Since the RHC produced at 350°C had the highest CEC, it was selected for the rice growing experiments.

Table 5.4 The CEC Capacities of RHC and MC (mean of 3 replicates ± standard error)

Chars	**CEC (meq kg^{-1})**
Miscanthus (350ºC pyrolysis)	36.6±0.54
Rice husk (350ºC pyrolysis)	81.2±0.13
Rice husk (700ºC pyrolysis)	17.1±0.38

5.3.2 Abiotic Assessment (E values) of the Feasibility of Using Biochar to Reduce Cd Bioavailability in the Contaminated Paddy Soils

Without biochar addition the E value for the medium-Cd contaminated soil was 41.0±2.03 mg kg^{-1} (Table 5.5). The E values for RHC and MC per se were 0.08±0.003 and 0.10±0.008 mg kg^{-1}, respectively, and so the char materials themselves contribute an environmentally insignificant amount of bioavailable Cd. E values of treatments were first calculated considering the total weight of solids (soil+char) ~1 g. In addition, the E values were re-calculated (or mass corrected) considering only the soil mass in each treatment, e.g. for the 10% char treatments, the E value was corrected for a soil mass of 0.9 g. Corrected E values of RHC and MC in medium and high-Cd soils are shown in Tables 5.5 and 5.6.

The E values, both on a total mass (soil+biochar) basis and when corrected for the soil mass in each treatment (i.e. soil only), showed reductions in available Cd when biochar was added to the medium-Cd contaminated soil (Table 5.7). With the amendment of RHC at 0-20%, the E values (corrected for soil mass in each treatment) were in the range 27.0 to 41.0 mg kg^{-1}, representing reductions of up to ~34% (Table 5.7). Addition of MC at 0-20% showed a very similar range (27.1-41.0 mg kg^{-1}, representing up to ~34.1% reduction; Table 5.7). The results indicate that 1% amendment with RHC reduced the E value (representing the available Cd) for the medium Cd soil by ~5%, while a 5% amendment reduced the E-value by 16%. Because the E values have been corrected for the soil mass in each treatment, these results clearly demonstrated that incorporation of biochar did not just reduce the E value through reducing the mass of soil in the solid material tested but rather it reduced the E-value by restricting the amount of Cd that was released from the soil into the solution. That is, the biochar reduced the bioavailability of the Cd in the medium Cd soil (Figure 5.10).

Beesley and Marmiroli (2011)* similarly found biochar to have a capacity to remove and retain metals from soil solution. They used a leaching column protocol to evaluate the efficacy and permanence of element (As, Cd and Zn) retention by biochar**. They found that Cd was quite high in eluate from an initial soil only column (200-500 μg L^{-1}) but that when this was passed through a biochar column the concentration in final eluate had decreased to less than 2 μg L^{-1}.

Considering the range 0-20% of chars added to medium-Cd contaminated soils, both RHC and MC had similar results at 20% char addition (~34% reductions, as discussed above) but the relationship between the amount of char added and the achieved reduction in availability was not linear for either char. At small percentages of added chars (1% and 5% chars added), RHC reduced the E value by 4.7% and 16%, respectively, while MC reduced it by just 2.1% and 3.8%, respectively. The greater effect of RHC per unit mass char added at lower addition rate led to the conclusion that RHC is the better option for remediating medium Cd-contaminated soils.

For the high-Cd contaminated soils, the E value for the unamended soil was 19.0±0.1 mg kg^{-1} (Table 5.8). As the amount of char increased from 0-20% w/w, the E values remained at ~18-19 mg kg^{-1} and so adding either of the two chars (0-20%) made no difference to the availability of Cd, in strong contrast to what had been observed for the medium Cd-contaminated soils (Table 5.7).

It seemed that char application had no effect on Cd availability in the high-Cd contaminated soils. It is possible that there are factors that inhibit the adsorption

* Soil was sourced from a canal embankment in Kidgrove, Staffordshire, UK. The origin of the pollutants in this soil is believed to be the discharge or spillage of contaminated effluent from neighbouring manufacturing industries.

** Biochar was produced by pyrolysis of hardwoods including Oak, Common Ash, Sycamore, birch and cherry at 400°C in steel furnaces (Bodfari Charcoal, Denbigh, North Wales, UK)

process between soils and chars such as the high amount of $CaCO_3$ and Zn in high-Cd contaminated soils. For the medium contaminated fields (F4/F14), Ca and Zn concentrations were ~4,000 mg kg^{-1} and ~750 mg kg^{-1}, respectively, whilst for the high contaminated fields (F10/F18), the concentrations were ~20,000 mg kg^{-1} and ~2,600 mg kg^{-1}, respectively. Thus, for the high contaminated soils, the Cd may have had to compete to a much greater extent with Ca and Zn for the sorption sites on the biochar.

Overall, the results suggest that a biochar amendment approach may be more effective at reducing bioavailable Cd in the medium-Cd contaminated soils but less effective for the high Cd soils. This is likely linked to the varying associations of Cd identified for these two soil groups in Chapters 3 and 4 (i.e. that the medium Cd soils had much higher relative and absolute amounts of readily exchangeable Cd while the high Cd soils had more carbonate bound Cd). In addition, the significantly higher concentrations of mining-derived Ca and Zn in the high contaminated soils may out-compete Cd for biochar sorption sites. These factors will be discussed further in the sections below.

Table 5.5 The E value of Medium Contaminated Soils (F14; 2nd Sampling, Total Cd 61.5 mg kg^{-1}) Mixed with Rice Husk Chars and Miscanthus Chars and the Corrected E Value (mean of duplicate ± standard error)

Chars added	Soil weight (g)	Rice husk chars		Miscanthus chars	
		E value (mg kg^{-1} soil+char)	E value corrected for soil mass	E value (mg kg^{-1} soil+char)	E value corrected for soil mass
0%	1	41.0±2.03	41.0±2.03	41.0±2.03	41.0±2.03
1%	0.99	38.7±0.65	39.1±0.65	39.8±0.88	40.2±0.88
5%	0.95	32.8±1.00	34.5±1.05	37.5±2.23	39.5±2.34
10%	0.9	28.5±1.22	31.6±1.36	30.2±0.14	33.6±0.16
15%	0.85	24.6±0.32	28.9±0.39	26.1±0.39	30.7±0.46
20%	0.8	21.6±0.48	27.0±0.64	21.7±0.83	27.1±1.02

Table 5.6 The E value of High Contaminated Soils (F10; total Cd 83.7 mg kg^{-1}) Mixed with Rice Husk Chars and Miscanthus Chars and the Corrected E Value (mean of duplicate ± standard error)

Chars added	Soil weight (g)	Rice husk chars		Miscanthus chars	
		E value (mg kg^{-1} soil+char)	E value corrected for soil mass	E value (mg kg^{-1} soil+char)	E value corrected for soil mass
0%	1	19.0±0.14	19.0±0.14	19.0±0.14	19.0±0.14
1%	0.99	19.1±0.50	19.4±0.51	19.7±0.55	19.2±0.50
5%	0.95	18.4±0.66	19.3±0.70	17.5.±0.60	18.5±0.63
10%	0.9	17.2±0.08	19.1±0.09	16.7±0.13	18.6±0.14
15%	0.85	16.8±0.69	19.8±0.82	16.2*	19.1*
20%	0.8	15.9±0.09	19.9±0.11	15.1±0.28	18.8±0.34

* n=1

Table 5.7 Changes in E value (corrected for soil mass) for Medium-Cd Soils (F14, 2nd Sampling, Total Cd 61.5 mg kg^{-1}) following amendment with RHC 350°C and MC 350°C (mean of duplicate ± standard error)

Chars	Rice husk chars		Miscanthus chars	
	E value (mg kg^{-1})	% reduction	E value (mg kg^{-1})	% reduction
0%	41.0±2.03	0.0%	41.0±2.03	0.0%
1%	39.1±0.65	4.7%	40.2±0.88	2.1%
5%	34.5±1.05	16.0%	39.5±2.34	3.8%
10%	31.6±1.36	22.9%	33.6±0.16	18.2%
15%	28.9±0.39	29.5%	30.7±0.46	25.2%
20%	27.0±0.64	34.3%	27.1±1.02	34.1%

Table 5.8 Changes in E Value (corrected for soil mass) for High-Cd Contaminated Soils (F10, total Cd 83.7 mg kg^{-1}) following Amendment with RHC 350°C and MC 350°C (mean of duplicate ± standard error)

Chars	Rice Husk Chars		Miscanthus Chars	
	E value (mg kg^{-1} soil)	% reduction	E value (mg kg^{-1} soil)	% reduction
0%	19.0±0.14	0.0%	19.0±0.14	0.0%
1%	19.3±0.51	-1.7%	19.9±0.50	-4.7%
5%	19.3±0.70	-1.7%	18.5±0.63	2.9%
10%	19.1±0.09	-0.6%	18.6±0.14	2.3%
15%	19.8±0.82	-3.8%	19.1*	-0.6%
20%	19.9±0.11	-4.4%	18.8±0.34	1.0%

* n=1

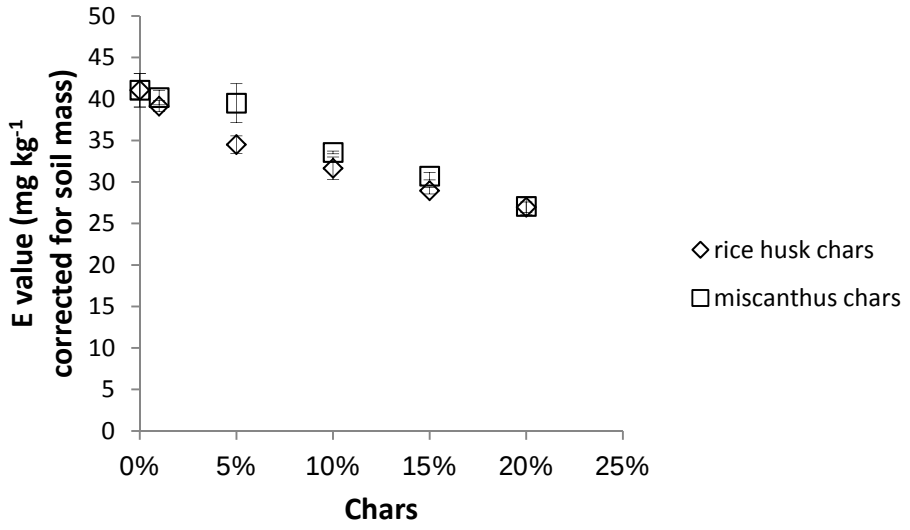


Figure 5.10 Changes in E value (corrected for soil mass) for the medium-Cd contaminated soils following amendment with RHC and MC; values are mean of duplicate ± standard error

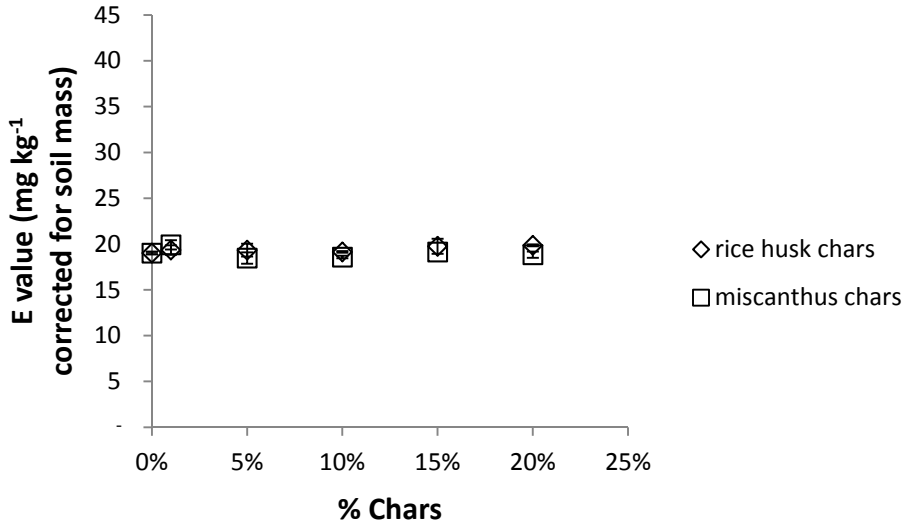


Figure 5.11 E values (corrected for soil mass) in the high-Cd contaminated soils following amendment with RHC and MC; values are mean of duplicate ± standard error

The extent of reduction of available Cd in the medium-Cd soils in this study was in the same range as that reported by Houben *et al.*, (2013)[*]. They used biochar (miscanthus straw[**]) as an amendment in contaminated soil and found a significant decrease in 0.01 M $CaCl_2$ extracted Cd concentration (which represented the available Cd). For 1, 5 and 10% w/w addition treatments, Houben *et al.*, (2013) observed 5, 20 and 35% reductions, respectively). In 5%- and 10%-biochar treatments, the Cd concentration in solution continued to slowly decrease over a test period of nearly two months, likely due to ageing reactions.

Another study involved high-Cd contaminated soils. Fellet *el at*. (2014) used three types of chars produced from: (i) pruning residues from orchards (ROM); (ii) fir tree pellets (ABE); (iii) manure pellets (70%) + fir tree pellets (30%) (MAN).

[*] They studied contaminated soils contain total Cd 24 mg kg^{-1} adjacent to a zinc and lead smelting plant in Sclaigneaus, Namur Province, Belgium.

[**] Miscanthus char was industrially produced in a commercial pyrolysis reactor by Pryreg GmbH (Dorth, Germany). The operating condition of production were a residence time in the reactor of 30 min and an end temperature of pyrolysis of 600 °C (Houben *et al.,* 2013).

These were applied at 1.5 and 3% w/w to remediate the contaminated soils investigated (total Cd 74.4 mg kg^{-1} from the mining site at Cave del Predil, NE Italy). The results showed that two treatments of the chars tested (MAN and ROM) significantly reduced the available Cd (extracted by DTPA). In comparison with the control, 53.7-82.4% reductions were achieved and 3%-MAN had the greatest capacity to sorb Cd (82.4%).

When compared to the present study, the work of Fellet and co-workers (2014) showed much higher reduction of available Cd in soils upon biochar addition (in particular by MAN). This may be attributable to differences in soil properties between the two studies and/or to the more vigorous extraction of Cd by DTPA compared with an E value measurement made using a neutral salt extraction. Alternatively, it could indicate that MAN has a greater Cd sorption capacity than RHC. These questions warrant further investigation in future.

5.3.3 Biotic Assessment (Rice Plants) of the Utility of Biochar for Restricting Cd Bioavailability in Paddy Soils and Comparison with E values and Tessier Extractions

5.3.3.1 Soil measurements (total Cd, E value, Tessier extractions) on soils from the rice growth study

For the medium Cd-contaminated soil prior to the rice growing experiments, the total Cd in the control treatment (without addition of RHC) was 22.6±0.44 mg kg^{-1} (Table 5.9), while the E value was 9.15±0.06 mg kg^{-1} (equalling 40.5% of total Cd (Table 5.10). This was consistent with the combination of soil from F4 and F14 which had E values of 6.92±0.07 and 14.5±0.16 mg kg^{-1}, respectively. For the Tessier scheme, the amount of Cd extracted during the first step (Tessier-exchangeable Cd) was 5.70±0.16 mg kg^{-1} (25.2% of total Cd) while that extracted

during the second step (Tessier-carbonate bound) was 7.92±0.20 mg kg^{-1} (35.0% of total Cd; Table 5.10). These values were slightly lower than expected based on those obtained for F4 (T1: 7.58±0.18 and T2: 7.62±0.52 mg kg^{-1}) and F14 (T1: 10.1±0.06 and T2: 16.4±0.38 mg kg^{-1}; see Table 3.15, Chapter 3).

For the high-Cd contaminated soils prior to the rice growing experiments, the total Cd in the control soils was 61.2±2.15 mg kg^{-1}(Table 5.9). The E value for these soils was 12.8±0.18 mg kg^{-1} (21.3% of total Cd), lower than expected from the combination of soils from F10 (19.0±0.14 mg kg^{-1}) and F18 (18.0±0.62 mg kg^{-1}). The Tessier-exchangeable Cd concentration was 9.28±0.09 mg kg^{-1} (15.2% of total Cd) while the Tessier-carbonate bound Cd was 48.5±0.65 mg kg^{-1} (79.2% of total Cd; Table 5.10). Again, these were lower than the values expected from the combination of F10 and F18. Nevertheless, the general trends were similar and the high Cd-contaminated soils contained lower proportions of exchangeable Cd and higher proportions of carbonate-bound Cd than the medium-contaminated soils.

Table 5.9 The Cd Concentration and the Reduction of Cd Level when Amended by RHC in the Medium and High-Cd Soils (pre- and post-rice growing period)*

Soil Type	Treatment	Total Cd (mg kg^{-1})		E value (mg kg^{-1})	Tessier exchangeable Cd (mg kg^{-1})		Tessier carbonate bound Cd (mg kg^{-1})	
		pre	post	pre	pre	post	pre	post
Medium-contaminated	0% RHC (n=6)	22.6±0.49[a] **	21.7±0.89	9.15±0.06[a]	5.70±0.16	6.09±0.33[a]	7.92±0.20	7.18±0.65
	1% RHC (n=3)	21.8±0.21[a]	21.0±0.22	8.83±0.11[b]	5.66±0.09	6.82±0.18[b]	7.43±0.05	4.95±0.30
	5% RHC (n=3)	21.2±0.40[b]	21.1 ±0.49	8.77±0.09[c]	5.61±0.09	5.71±0.03[a]	7.76±0.05	7.76±0.06
		p=0.004	p=0.204	p<0.001	p=0.074	p=0.004	p=0.07	p=0.074
High-contaminated	0% RHC (n=6)	61.2±2.15	59.5±1.7	12.8±0.18	9.28±0.09[a]	8.63±0.29	48.5±0.65	48.6±1.4
	1% RHC (n=3)	64.1±1.15	56.9±0.81	13.2±0.05	9.74±0.07[b]	9.09±0.18	47.6±1.0	48.0±3.4
	5% RHC (n=3)	62.4±1.89	61.3±3.0	13.0±0.19	9.67±0.20[b]	9.04±0.34	44.2±1.8	43.6±0.59
		p=0.349	p=0.441	p=0.333	p=0.017	p=0.660	p=0.910	p=0.061

* All values are corrected for the mass of soil; mean ± standard error shown

** The results of the least significant difference (LSD) tested within each group; the same letter after the value indicates no significant difference.

Table 5.10 The % Change in Total Cd Concentration and E values, Exchangeable and Carbonate-bound Cd Expressed as a % of Total Cd Concentration Prior to and After Rice Growth in Control and RHC-treated Medium- and High-contaminated Paddy Soils (mean ± standard error)

Soil Type	Treatment	% change in total Cd	E value as % of total Cd	Tessier exchangeable Cd as % of total Cd		Tessier carbonate bound Cd as % of total Cd	
			Pre	Pre	post	pre	post
Medium-contaminated	0% RHC (n=6)	-4.0	40.5±0.92	25.2±0.91	28.1±1.9	35.1±0.88	33.1±2.0
	1% RHC (n=3)	-3.9	40.4±0.64	25.9±0.48	32.5±0.90	34.0±0.51	23.6±0.87
	5% RHC (n=3)	-0.6	41.3±0.88	26.4±0.65	27.1±0.65	36.6±0.69	36.8±0.87
High-contaminated	0% RHC (n=6)	-2.8	20.9±0.79	15.2±0.55	14.5±0.64	79.2±2.8	81.6±2.4
	1% RHC (n=3)	-11.2	20.5±0.38	15.2±0.30	16.0±0.39	74.2±1.4	84.2±1.2
	5% RHC (n=3)	-1.8	20.9±0.70	15.5±0.57	14.7±0.92	70.8±2.2	71.0±3.5

The reduction of available Cd concentration in soils when amended by chars (prior to rice growing)

- Medium-Cd contaminated soil

The values for total Cd concentration in soils which had 0%, 1% and 5% char significant decreased in the pre-rice growing period after correction for the total mass of soil (22.6±0.49, 21.84±0.21 and 21.22±0.40 mg kg^{-1}, respectively). The E values measured for these soils also significantly decreased ($p=0.004$) in the pre-rice growing period; that is 9.15±0.06, 8.83±0.11 and 8.77±0.09 mg kg^{-1}, respectively, corresponding to 40.5, 40.4 and 41.3% of total Cd, respectively (Tables 5.9 and 5.10). Thus, unlike the work presented in Section 5.3.2 (cf ~16% reduction for 5% char addition; Table 5.7), there was no discernible reduction of Cd availability as a % of total Cd as a result of biochar addition. The reason for this is

not clear and warrants further investigation in terms of the consistency with which biochar can decrease Cd availability in soil. Since a new batch of RHC was used for these experiments, one avenue of exploration should be the between-batch variability of char properties.

Tessier-exchangeable Cd concentrations were 5.66±0.09 mg kg^{-1} and 5.61±0.09 mg kg^{-1} for the 1% and 5% RHC treatments, respectively. There was no significant difference between the treatments.. When expressed as a % of total soil Cd (Table 5.10), it was clear that there was no discernible change with the addition of RHC. Similarly, the Tessier carbonate-bound Cd concentrations did not differ significantly with the addition of char; p=0.07 (Tables 5.9 and 5.10).

- High-Cd contaminated soils

The values for total Cd concentration in soils which had 0%, 1% and 5% char were 61.2±2.15, 64.2±1.15 and 62.4±1.89 mg kg^{-1}, respectively, after correction for the total mass of soil (Table 5.9). The addition of char had no significant effect on soil total Cd concentration.

The E values for the 1% and 5% RHC treatments were 13.2±0.05 and 13.0±0.19 mg kg^{-1} which were not significantly different from the control (12.8±0.18 mg kg^{-1}; p=0.333) (Table 5.9). When expressed as a % of total Cd, the E values were 20.9±0.79, 20.5±0.38 and 20.5±0.38% and so the addition of RHC had no discernible effect on the availability of Cd as determined by the stable isotope method (Table 5.10).

As for the E values, for the 1% and 5% RHC treatments, the Tessier-exchangeable Cd concentration (9.74±0.07 and 9.67±0.20 mg kg^{-1}) were significantly different from the control value (9.28±0.09 mg kg^{-1}; p=0.017) (Table 5.9). In contrast,

Tessier-carbonate bound Cd for the 5% RHC treatment (42.0±1.76 mg kg^{-1}; p=0.910) was not statistically significantly lower than the value for the control.

The reduction of Cd concentrations in soils after rice growing

Table 5.10 shows that there was generally a decrease (0.6-11.2%) in total Cd concentrations after the rice growing experiments. However, the decreases were generally small as might be expected even if plant uptake has taken place.

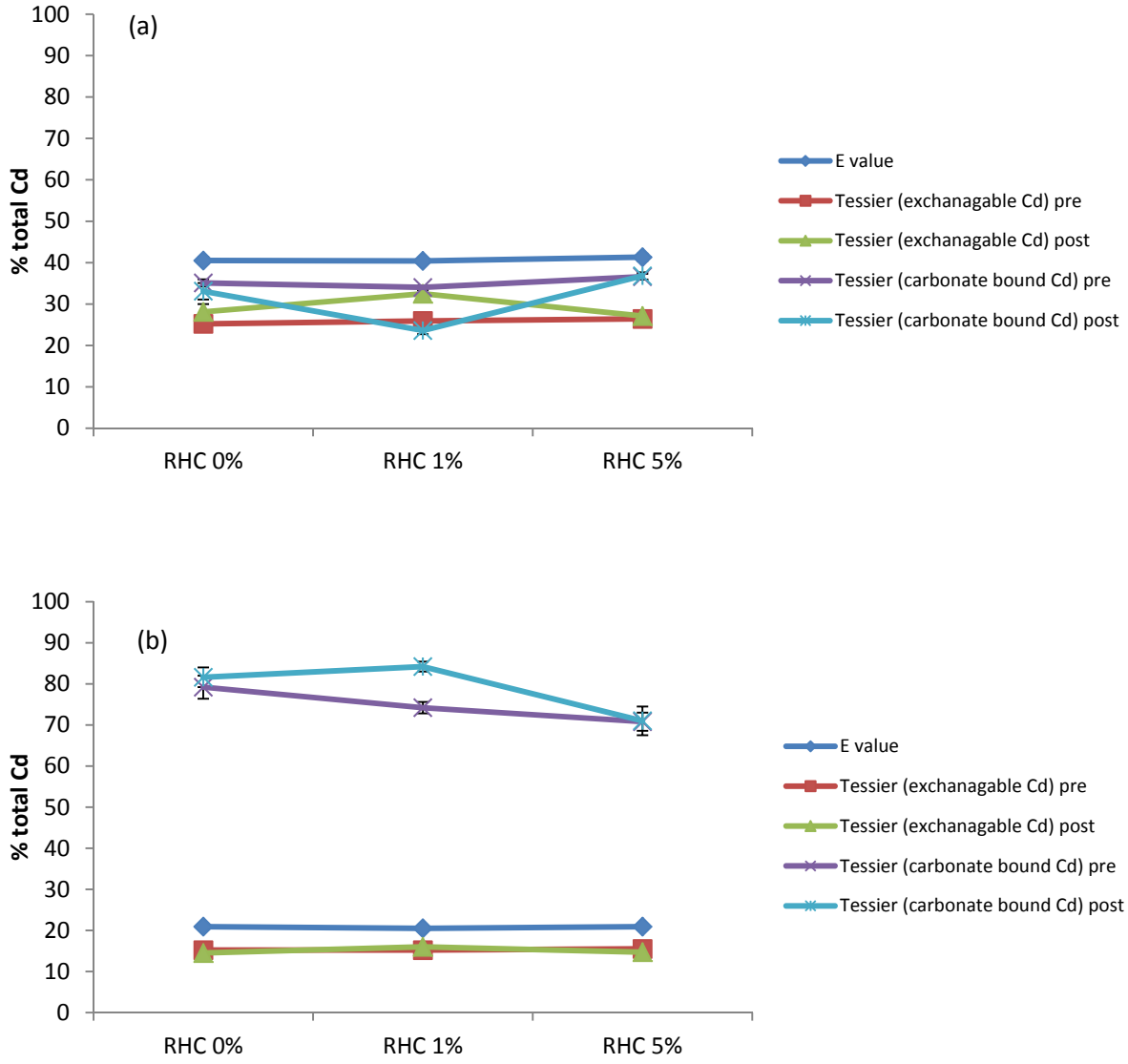


Figure 5.12 E values, exchangeable and carbonate-bound Cd expressed as a % of total Cd prior to and post rice growth experiments in control and RHC-amended (a) medium- and (b) high-contaminated paddy soils (mean ± standard error)

- Medium-Cd contaminated soil

After the rice growing experiments, Figure 5.12(a) illustrates that there was no change in Tessier exchangeable and carbonate-bound Cd concentrations as a % of total soil Cd in the control soil and in the 5% RHC-treated soil in comparison with the initial values. For the 1% RHC treatment, there was a statistically significant increase in exchangeable Cd ($p=0.004$) whilst the decrease in carbonate-bound Cd was not significant ($p=0.074$). However with limited replicates, these experiments would need to be repeated to establish whether or not a speciation change had resulted from growing rice in the biochar-amended soil.

- high-Cd contaminated soil

Figure 5.12(b) showed that there was no discernible change in Tessier-exchangeable Cd (not statistical significant decrease; $p=0.660$) after rice growth for the control soil and for both RHC treatments. As for the pre-growth experiments, however, there was a decrease (but not significantly; $p=0.910$) in carbonate-bound Cd for the 5% RHC treatment in comparison with the control soil. There was no effect on the E value and so it is proposed that this biochar-induced change in Cd speciation has no effect on the availability of Cd. This hypothesis will be examined further in the sections which follow.

5.3.3.2 Rice Plant Results

For the rice grown on the medium-Cd contaminated soils, the root Cd concentrations were 43.3±1.48, 32.3±5.15 and 30.2±4.70 mg kg^{-1} in the control (0%), 1% and 5% RHC treatment, respectively (Table 5.11). These values were higher than the root concentrations obtained for rice grown in the unamended soils in the fields (F4 and F14; 24.8±0.43 and 16.7±0.12 mg kg^{-1} from Table 3.17, Chapter 3). It is possible that the conditions adopted for the pot experiments may have resulted in higher Cd absorption by rice roots. Nevertheless, there was a much decrease but not significant in rice root concentration with increasing biochar

content of the medium Cd-contaminated soil. In contrast, for the high-Cd contaminated soils, the root Cd concentrations were 53.1±2.90, 49.7±4.13 and 66.9±20.2 mg kg^{-1} in the control (0%), 1% and 5% RHC treatments, respectively (Table 5.11). In a reversal of what was noted in the medium Cd soils, the high Cd soils showed root Cd concentrations that were lower than those measured for rice grown in the field on these high Cd soils (F10 and F18; 131.5 ±1.95 and 86.1±.2.13 mg kg^{-1} from Chapter 3). Moreover, for these high-Cd soils, there was no decrease in root Cd content with increasing char content of the soil.

In rice straw, the Cd concentration from medium Cd-contaminated soils ranged from 0.98 to 3.40 mg kg^{-1} (Table 5.11). As for the rice roots, these values were much higher than the straw concentration in rice grown in the field (i.e. F4 and F14 had 0.27±0.02 and 0.14±0.01 mg kg^{-1}, respectively; Chapter 3). In the high-Cd contaminated soils the straw Cd concentration ranged from 1.84 to 2.19 mg kg^{-1} (Table 5.11), which was just slightly lower than the range observed for rice grown in the field on these soils (F10 and F18; 2.39±0.17 and 3.10±0.49 mg kg^{-1} respectively; Chapter 3).

Table 5.11 The Cd Concentration in Rice when Amended by RHC (mean ± standard error)

Soils	Rich Husk Chars (%)	Cd concentration (mg kg^{-1})	
		Root	Straw
medium-Cd contaminated soils	Control (0%) (n=3) (n=4)	43.3±1.48	2.88±0.55
	1% (n=3)	32.3±5.15 (25.4%)*	3.40±0.57 (-18.0%)
	5% (n=3) (n=2)	30.2±4.70 (30.4%)	0.98±0.08 (66.1%)
		p=0.128	p=0.078
high-Cd contaminated soils	Control (0%) (n=4)	53.1±2.90	2.02±0.49
	1% (n=2)	49.7±4.13 (6.3%)	1.84±0.19 (8.9%)
	5% (n=2)	66.9±20.2 (-26.1%)	2.19±0.20 (-8.5%)
		p=0.371	p=0.942

* Values in parentheses give the percentage reduction from the control (0% char)

Calculation of ratio values for the pot experiments showed that the soil-plant transfer coefficients (ratio of root : soil) and the translocated coefficients (ratio of straw : root) were not clearly different to in the fields (Table 5.12). In the medium-Cd soils the ratio of root : soil (fields) was 1.48 and 0.47 (F4 and F14, respectively) which was lower than in the pots (1.92, 1.50 and 1.50 in the control, 1% RHC and 5% RHC treatment, respectively). Though perhaps not statistically different, it was encouraging to note that the highest ratio was found in the control treatment (i.e. suggesting a possible ability for biochar to reduce soil-root transfer). The ratio of straw : root (fields) was 0.02 and 0.004 (F4 and F14, respectively) which was also lower than in the pots (0.07, 0.11 and 0.03 in the control, 1% RHC and 5% RHC treatment, respectively).

In the high-Cd soils, the ratio of root : soil (fields) was 1.57 and 1.05 (F4 and F14, respectively) which was higher than in the pots (0.87, 0.78 and 1.13 in the control, 1% RHC and 5% RHC treatment, respectively). The ratio straw : root (fields) was 0.03 and 0.04 (F4 and F14, respectively) which were very similar to those in the

pots (0.04, 0.04 and 0.03 in the control, 1% RHC and 5% RHC treatment, respectively). These results indicate that, for the medium-Cd soils examined, Cd was absorbed from soil to root to a greater extent in the pots than observed in the fields and also that Cd can be mobilised to the upper parts of rice more readily. For the high-Cd soils, however, the Cd was transferred from soil to root to a lesser extent in the pots compared to in the field. Another contrasting finding between the soils was that while the soil : root transfer ratios were possibly decreased by the presence of biochar in the medium-Cd soils, this was not evident in the high-Cd soils (Table 5.12).

Table 5.12 Comparison of the Ratio of Cd Concentrations in Root : Soil and Straw : Root between in the Fields and in the Pots

		Ratio root : soil	Ratio straw : root
Fields			
The medium-Cd contaminated soils	F4	1.48	0.02
	F14	0.47	0.004
The high-Cd contaminated soils	F10	1.57	0.03
	F18	1.05	0.04
Pots			
The medium-Cd contaminated soils +RHC treatment	0%	1.92	0.07
	1%	1.50	0.11
	5%	1.50	0.03
The high-Cd contaminated soils + RHC treatment	0%	0.87	0.04
	1%	0.78	0.04
	5%	1.13	0.03

The pot experiment thus showed assimilation of Cd by the plants from the soil but, for some soils and plant parts, this was to a different extent to that observed in the field in Thailand.

Such differences, e.g. higher root uptake in the pot experiments, may be attributable to the higher Tessier exchangeable value in pot soils after growing rice in the 1% RHC treatment or to differences in growth period, pot effects (i.e. different root restriction compared with in the field), or to differences in other conditions that could not be created in the laboratory. Nevertheless, because Cd was transferred from soil to plant in the laboratory study the success of biochar treatment in restricting that uptake can be evaluated.

The root Cd in the medium-Cd contaminated soils decreased 25.4% and 30.4% in the 1% and 5% RHC treatment, respectively (Table 5.11; Figure 5.13), suggesting that biochar addition reduced Cd uptake by rice in these soils. However, the straw Cd concentration showed variable results, nominally increasing 18% in 1% RHC amendment but decreasing 66.1% in 5% RHC treatment relative to the un-amended soils. Analysis of variance (ANOVA) of these results showed that in the medium-Cd contaminated soils, there was no significant difference among the control, 1% and 5% RHC treatment ($p=0.120$). But the least significant difference (LSD) analysis showed a very nearly significant difference of the means between control treatment and 5% RHC treatment ($p=0.070$). For the straw Cd, the ANOVA also showed no significant difference of straw Cd concentration among the control, 1% and 5% RHC treatment ($p=0.078$). But the LSD analysis showed the significant difference of the means between the 1% and 5% RHC treatment ($p=0.034$). Moreover the difference of the means between the control and 5% treatment was very nearly significant ($p=0.063$). Having found nearly significant differences between some treatments could be because the effect of the char is not great enough or because the variability for some treatments was too great, i.e. high standard errors, to allow differences to be discernible. Thus, suggestions here for future work could include: (i) addition of a greater amount of char to produce measurable effects and (ii) use of a greater number of replicates to establish statistically significant differences.

For the high-Cd contaminated soils, the apparently higher root Cd concentrations (26.1% compared to the control) in the 5% RHC treated soils were not statistically different (ANOVA) from those for the roots of the control plants. This was attributable to the high standard error on the mean of the two replicates (Table 5.11). Thus, no interpretation can be made in relation to the effect of (i) biochar on root Cd uptake; (ii) biochar-induced change in Cd speciation root Cd uptake. Thus the discussion below focuses on published studies involving medium-contaminated soils.

In a similar concentration range to the medium-Cd contaminated soils examined in the present study, Houben *et al*. (2013) found that miscanthus chars could be used to ameliorate Cd contaminated soil (adjacent to a zinc and lead smelting plant in Sclaigneaus, Namur province, Belgium; total Cd 24 mg kg^{-1}). Experiments investigating Cd uptake in ryegrass grown in the contaminated soil showed that for shoots, the 1% biochar treatment did not generate a difference to the control but the 5% biochar treatment did reduce uptake by 40% and 53% (for the first and second harvest, respectively) relative to the control. Indeed, 10% biochar treatment reduced shoot Cd uptake by 67% and 75%. This was in agreement with the decrease observed in 0.01M $CaCl_2$ extracted soil Cd concentrations (representing an abiotic measure of available Cd) from the control of 5, 20 and 35% (for 1%, 5% and 10% treatments, respectively) after one hour incubation. The authors also found that (i) the increase of soil pH due to char addition can reduce the amount of available Cd (ii) the effectiveness of biochar was reduced by ageing, i.e. root Cd concentration in the second harvest (age 8 weeks) was higher than in the first harvest (age 4 weeks).

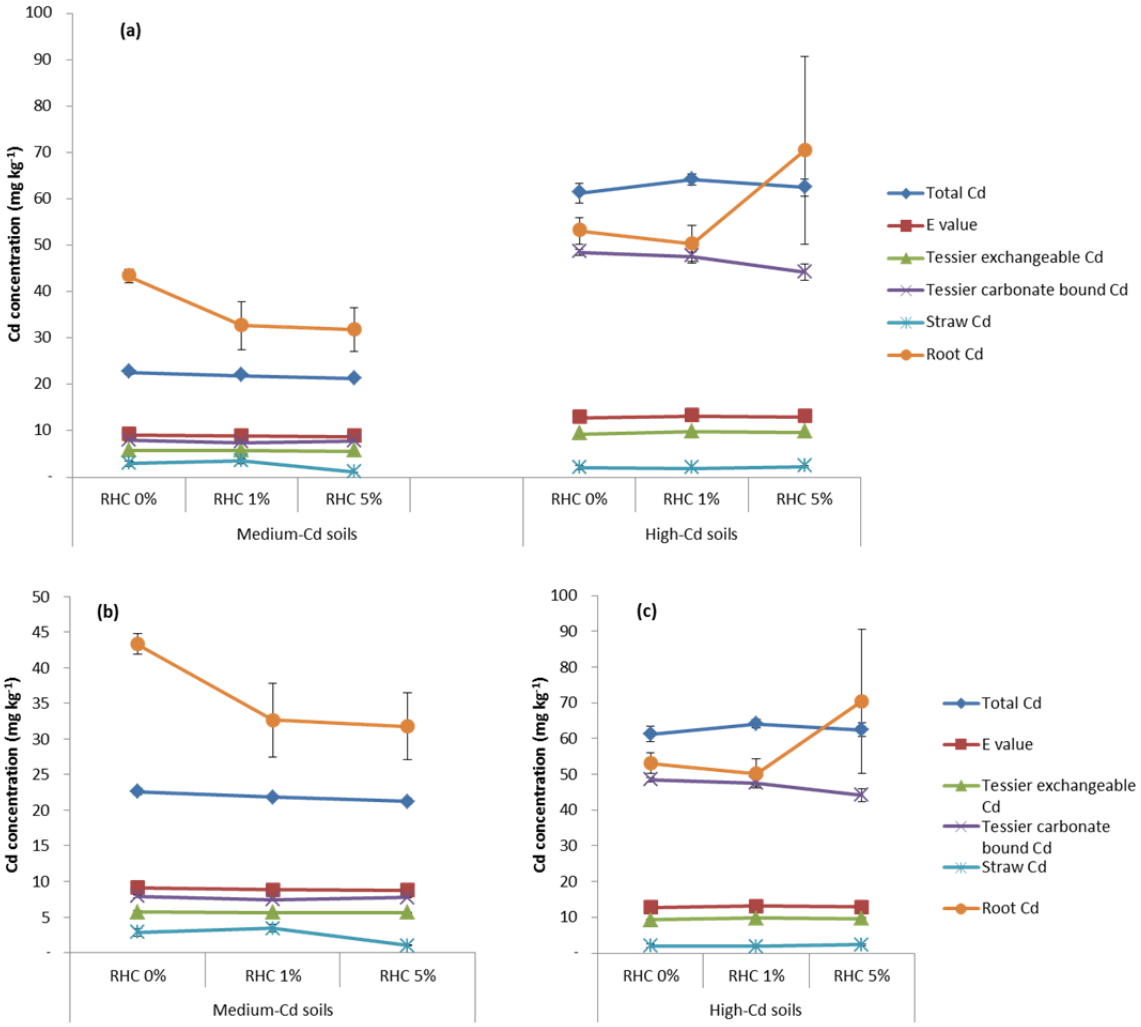


Figure 5.13 Concentration with standard error of the Root and Straw Cd in comparison with total Cd, E values the first two-Tessier scheme extractions (measured prior to the growing experiment) in medium and high Cd-contaminated soils pre- and post- rice growing; (a) both medium and high-Cd contaminated soils (b) only medium-Cd contaminated soils and (c) only high-Cd contaminated soils

5.4 Conclusions

From the characterisation of biochars used in this study, SEM analysis confirmed the porous structure of RHC 700ºC with large pores of ~20 µm and small pores in the 2-3 µm size range. RHC and MC produced at 350ºC had similar functional groups and both had more carboxyl and carbonyl functional groups than RHC

produced at 700 ºC. In addition, RHC (350ºC) had a significantly higher CEC capacity than both MC (350ºC) and RHC (700ºC). The feasibility study involving RHC and MC (both produced at 350°C) amendment of medium and high-contaminated soils (i.e. the abiotic study) found that both were effective at reducing bioavailable Cd (E values) in the medium-Cd contaminated soils (~34% for a 20% addition of either char) but had no effect on Cd availability in the high Cd-contaminated soils. Since the highly contaminated soils had significantly elevated Ca and Zn concentrations, this may be due to competition between Cd and Ca and/or Zn for the exchange sites on the biochar. Further laboratory experiments would be needed to verify this hypothesis.

Evaluation of E values for the soils used for the rice growth experiments, showed no effect of biochar addition for 1% and 5% RHC addition to either medium- and high-Cd contaminated soils. This was in strong contrast with the abiotic experiments and a focus for future investigation should be the between-batch variability in char properties as this was the main difference between the abiotic and biotic experiments.

Very little difference in Cd speciation was observed for the post- compared with the pre-rice growth experiments. The only significant difference was a decrease in carbonate-bound Cd for the 5% RHC treatment of the high Cd-contaminated soil in comparison with the control for this soil type. Since there was no effect on the E value, it was postulated that this change would have no effect on availability of Cd for plant uptake.

Analysis of the rice plants demonstrated that there were significant differences in the extent of uptake observed for the pot experiments compared with the field-grown rice (Chapter 3). The differences were not, however, the same for the medium- and high-contaminated fields. Specifically, in comparison with the field-grown rice, greater and lesser extents of uptake by roots were observed for the medium-and high-contaminated pot soils, respectively. Thus further work needs to

be done to better simulate field conditions within the laboratory. Nevertheless, one encouraging result was that, in spite of the lack of change in Cd speciation, there was apparent decrease in the extent of root uptake of Cd with increasing biochar addition for the medium-contaminated soils.

Finally, it is proposed that further work should involve addition of greater amounts of biochar (e.g. perhaps 5%, 10% and 20%) and the use of a greater number of replicates to produce measurable and statistically significant differences between the control and treatments.

References

Basu, P. (2013) Biomass Gasification, Pyrolysis, and Torrefaction: Practical Design and Theory. Second Edition. London. Elsevier. 147-176.

Beesley, L., E. Moreno-Jimenez, J.L. Gomez-Eyles, E. Harris, B. Robinson and T. Sizmur. (2011) A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environmental Pollution* 159 (12): 3269-3282.

Beesley, L and M. Marmiroli. (2011) The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. *Environmental Pollution* 159(2): 474-480.

Chen, B., Z. Chen and S. Lv. (2011) A novel magnetic biochar efficiently sorbs organic pollutants and phosphate. *Biosource Technology* 102(2): 716-723.

Downie, A.E., L.V. Zwieten, R.J. Smernik, S. Morris and P.R. Munroe. (2011) Terra Preta Australis: Reassessing the carbon storage capacity of temperate soils. *Agriculture, Ecosystems and Environment* 140(1-2): 137-147.

EPA. (2002) Guidelines for the Application of SEM/EDX Analytical Techniques to Particulate Matter Samples. pp. 91.

Fellet, G., M. Marmiroli and L. Marchiol. (2014) Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. *Science of the Total Environment* 468–469: 598-608.

Houben, D., L. Evrard L and P. Sonnet. (2013) Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere* 92(11): 1450-1457.

Huang, M., L. Yang, H. Qin, L. Jiang and Y. Zou. (2013) Quantifying the effect of biochar amendment on soil quality and crop productivity in Chinese rice paddies. *Field Crops Research* 154, 172-177.

Kaewchumnong, K and A.H. Price. 2008. A study on the susceptibility of rice cultivars to *Striga Hermonthica* and mapping of Striga tolerance quantitative trait loci in rice. *New Phytologist* 180: 206-216.

Kookana, R.S., X-Y. Yu and G-G. Ying. (2010) "Black is the new green": the blue shades of biochar. In '19th World Congress of Soil Science, Soil Solutions for a Changing World', Brisbane, Australia.

Kosolsaksakul, P., J.G. Farmer, I.W. Oliver and M.C. Graham. (2014) Geochemical associations and availability of cadmium (Cd) in a paddy field system, northwestern Thailand. *Environmental Pollution* 187: 153-161.

Lehmann, J. and S. Joseph. (2009) Biochar for Environmental Management. Chapter 1 in: Lehmann, J. and S. Joseph (Eds), *Biochar for Environmental Management: Science and Technology*. Routledge, London.

Mao, J.D., R.L. Johnson, J. Lehmann, D.C. Olk, E.G. Neves, M.L. Thompsonand K. Schmidt-Rohr. (2012) Abundant and Stable Char Residues in Soils: Implications for Soil Fertility and Carbon Sequestration. *Environmental Science & Technology* 46(17): 9571-9576.

Martin, S.M., R.S. Kookana, L. Van Zwieten and E. Krull. (2012) Marked changes in herbicide sorption–desorption upon ageing of biochars in soil. *Journal of Hazardous Materials* 231–232: 70-78.

McHenry, M.P. (2009) Agricultural bio-char production, renewable energy generation and farm carbon sequestration in Western Australia: Certainty, uncertainty and risk. *Agriculture, Ecosystems & Environment* 129(1-3): 1-7.

Paz-Ferreiro, J., H. Lu, S. Fu, A. Méndez, and G. Gascó. (2014) Use of phytoremediation and biochar to remediate heavy metal polluted soils: a review. *Solid Earth* 5: 65–75.

Sohi, S., E. Lopez-Capel, E. Krull and R. Bol. (2009) Biochar, climate change and soil: A review to guide future research. CSIRO Land and Water Science Report 05/09. Australia.

Xu. G., J.N. Sun, H.B. Shao and S.X. Chang. (2014) Biochar had effects on phosphorus sorption and desorption in three soils with differing acidity. *Ecological Engineering* 62: 54-60.

Zheng, H., Z. Wang, X. Deng, S. Herbert and B. Xing. (2013) Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. *Geoderma* 206: 32-39.

Chapter 6 Synthesis, Final Conclusions and Further Work

This chapter brings together the key results from chapters 3, 4 and 5 in order to: (i) demonstrate how the research objectives have been met; (ii) explore the implications of the research findings for future work in Thailand; and (iii) explore the wider implications of the research findings for managing Cd-contaminated soils and soil-food chain transfer worldwide.

More specifically:

- Sections 6.1-6.3 address the first two objectives which were to:
  - characterise the mineral form of Cd present in the mining waste and ore material and any transformations in chemical form occurring during riverine transport of the material as suspended particulate matter.
  - elucidate the geochemical transformations of Cd within the community irrigation system and upon transfer to the paddy field soils;
- Sections 6.4-6.7 address the final two objectives, to:
  (i) establish links between the solid phase Cd speciation and Cd availability to rice plants by using bioavailability assessment methods e.g. Tessier sequential extraction, BCR extraction and a stable isotope exchange technique;
  (ii) trial selected soil amendments in laboratory experiments to reduce Cd availability to rice plants.
- Sections 6.6-6.7 also consider the implications of the experimental results involving biochar addition for future work in Thailand.
- Section 6.8 tackles the wider implications of the research findings for future work further afield.

6.1 Mining area – Elemental Concentrations and Mineralogy

Although several mining companies had been operational in recent decades, currently only the Padaeng Company extracts Zn from the ore materials present in the Padaeng deposit in the mountainous area to the east of Mae Sot. The proxy ore sample obtained in this study contained ~11.7% w/w Zn and ~0.3% w/w Cd as well as 2.6% w/w Fe, ~430 mg kg^{-1} Pb and 0.09% w/w S. The main Zn-containing ores were identified as smithsonite ($ZnCO_3$ ~12.5% w/w) and hemimorphite ($Zn_4Si_2O_7$ $(OH)_2$ ~3.1% w/w), consistent with the previously reported geology of the area. Specifically, secondary deposits of these minerals within colloform bands are known to have formed from weathering of sulphide materials including galena (PbS), pyrite (FeS_2), and sphalerite (ZnS). Other major minerals present in the proxy ore sample were calcite ($CaCO_3$ ~0.3% w/w), dolomite ($CaMgCO_3$ ~16% w/w) and the phyllosilicates, clinochlore (14.1% w/w), kaolinite (2.3% w/w) and chlorite (4.5% w/w). Characterisation of this sample provided a useful comparison with the mineralogy of the soils and sediments downstream of the mining area. Waste storage procedures are reportedly much improved and aqueous phase Cd concentrations downstream of the Padaeng mining site have dropped considerably in recent years (see introduction, Chapter 3). However, during the flood period, we observed that red soil material was being transported from the mountainous area via the Mae Tao Creek through the community irrigation system to the paddy fields in Mae Sot and so both the aqueous phase and the suspended sediment components of creek and canal waters were investigated in this study especially during the flood period.

6.2 Creek and Canal – Water (pH/redox) and Sediment (Particle Size) Properties, Elemental Concentrations, Elemental Associations and Mineralogy

During all three sampling periods (dry, flood and harvest period), the creek and canal waters were slightly alkaline (pH 7.6-8.0) consistent with the influence of the underlying carbonate-rich sediments (~5.0-6.0% w/w calcite; ~2.5-4.3% w/w dolomite). In contrast, the redox status of the waters showed seasonal variability. The

waters became increasingly anoxic from the dry season (~350 mV) through the flood period (~250 mV) to the harvest period (~150 mV). This transition coincided with the increase in temperature and humidity from ~23°C in winter to ~29°C in summer and is therefore attributable to the enhanced breakdown of organic matter within the water column especially towards harvest time when water flow is markedly reduced.

Particle size analysis of the creek and canal sediments showed that the largest proportion (48-72%) of particles were in the 20-53 µm size range with a further 11-21% in the <20 µm size range. XRPD analysis showed that both size fractions contained the carbonates mentioned above and, in addition, the <20 µm fraction contained significant amounts of muscovite and chlorite minerals. Consistent with the coarser nature of particles within the 20-53 µm fraction, there were no detectable clay minerals but instead a higher proportion of quartz minerals (~91% vs 67% for <20 µm fraction). Although the composition of the suspended particles from the overlying canal water was more similar to that of the <20 µm sediment size fraction, there were some obvious differences in mineralogy. For example, the suspended particles comprised ~31% illite and only ~0.3% muscovite whilst the <20 µm sediment size fraction contained no detectable illite but ~20% muscovite. Interestingly, illite has a structure similar to and indeed is a weathering product of muscovite.

In accordance with the reduced impact of mining activities upstream, Cd concentrations in the 0.2 µm-filtered creek and canal waters was typically low (<0.1 µg L^{-1}) except during the flood period when values of 0.5-6.2 µg L^{-1} were obtained. All values were significantly less than the Thai safe level for consumers; 5 µg L^{-1} (where water hardness is >100 mg L^{-1}) for the surface water quality standards, and most were below the WHO standard for drinking water of 5 µg L^{-1}. Suspended particulate Cd was only detected during the flood period with concentrations in the range 4.2-9.7 µg L^{-1} and comprised >80% total Cd (suspended+particulate) concentrations in the canal waters. Moreover, during the flood period, total Cd

concentrations decreased with increasing distance from the main creek through the canal towards the paddy fields and this most likely reflects removal to the solid phase via settling out of particulate matter in the shallower canal waters and/or via precipitation. In support was the observation that sediment Cd concentrations were higher (up to 27 mg kg^{-1}) downstream of the main check dam than upstream of it (15-16 mg kg^{-1}) and from communications with the local farmers, the canal is dredged on an annual basis to clear the build-up of sediment.

With respect to transfer of Cd to the paddy fields, Cd in the standing waters obtained during the flood period was almost entirely associated with suspended particulate matter and so it was concluded that, despite recent improvements in mining practices, e.g. the construction of additional emergency ponds to contain storm water in the mining area and the construction of dykes around its perimeter to prevent soil erosion (Padaeng Industry Public Company Limited, 2007), the Mae Tao creek is still transferring Cd, albeit at a reduced rate, from the mining area and mainly in association with suspended particulate matter, to the paddy fields.

Further analysis of the canal sediments showed that 25-55% of Cd was in the 20-53 µm fraction and 24-52% was in the <20 µm fraction while <31% was present in the >53 µm size fraction. Thus, as expected, Cd was predominantly associated with the finer-sized sediment particles. Tessier sequential extraction then showed that there was a difference in association of Cd within the <20 µm compared with the 20-53 µm size fraction. Specifically, there was a greater proportion associated with carbonate in the coarser (~44%) compared with the finer (~17%) size fraction whilst the opposite trend was observed for exchangeable Cd (coarser: 3% vs finer: ~36%). SEM-EDX analysis confirmed Cd associations with carbonates within the 20-53 µm size fraction. In some particles, Cd-clay interactions were also detected. Thus it can be concluded that, as a consequence of the annual dredging of the canal sediments, carbonate-bound and clay-bound Cd is being transferred to the paddy fields.

6.3 Paddy Fields – Water flow, Water and Soil (pH/redox/OM/moisture/particle size) Properties, Elemental Concentrations and Associations, Transfer Pathways from Source to Fields, Depth of Contamination

From discussion with the local farmers it was established that water enters the paddy fields at the study site through F1 and F3 and flows into F2, 4, 5 and 6, then to F6-9, F10-13 and finally F14-18. F14-16 are the lowest lying fields and the main outflow is via F15. The pH of the soils decreases from ~7.7 in F1 through to ~7.2 in F7, consistent with a decreasing influence of carbonate-rich particulate matter entering the fields from the canal. The pH values for the rest of the fields vary between 6.8 and 7.8 and, although there is no definitive information, the higher values may reflect an influence of liming (personal communication with local farmers). There was no obvious trend in organic matter content but highest moisture contents were obtained for the lowest lying fields during both the dry and harvest periods.

Particle size analysis showed that the fields closest to the inlet (F1 and F7) had a finer texture in comparison with those furthest away (F10 and F18) and this probably reflects the extent of deposition of the fine canal sediments in the former. Although F10 and F18 have been artificially raised using topsoil from the fields closest to the inlet, the paddy soils are mixed to a depth of 40 cm and so the finer composition of the added soil would not necessarily influence the overall composition of F10 and F18.

The pattern of Cd contamination across the field group did not reflect what might have been expected from previous work (e.g. Simmons *et al.*, 2005) where highest concentrations were obtained for soils closest to the creeks and canals and decreased with increasing distance inland. Indeed, low concentrations (<10 mg kg^{-1}) were obtained for the fields closest to the inlet and the highest values were obtained for the

lowest lying fields which, as mentioned above, had been artificially raised with topsoil from the fields closest to the inlet. Thus it was concluded that heavily Cd-contaminated soils had been transferred to the low lying fields. The soil profiles confirmed this spatial distribution pattern and also showed that the contamination was detectable to a depth of ~40 cm, consistent with the depth to which the soil is mixed in preparation for rice-growing. In addition, analysis of fertilizers applied to these fields confirmed that they contained insignificant amounts of Cd and so could not account for the extent of contamination observed in these paddy fields

The wider elemental analysis of the paddy soils revealed similar spatial patterns for Zn, Ca and S as for Cd. Since these elements were present at high concentrations in the ore proxy sample, this provides further evidence for the transfer of mining-derived material onto the paddy fields. XRPD analysis of the heavily contaminated soils from F10 and F18 showed that they both had very high proportions (~85-86%) of quartz in the 20-53 μm fraction whilst the <20 μm fraction had greater amounts of carbonate minerals (~17-19%) and 42-46% illite. The latter was most similar to the composition of suspended particulate matter in the canal water. As for the canal sediments, SEM-EDX identified both carbonate- and clay-bound forms of Cd. Thus the pathways by which Cd is introduced to the paddy fields are:

(i) deposition of suspended particulate matter during periods of irrigation;
(ii) deposition of sediments following annual dredging of the canal;
(iii) one-off transfer of soil from higher lying to lower lying fields.

It is important to recognize the importance of local knowledge in establishing these transfer pathways and in explaining the spatial and vertical distribution of Cd within the paddy fields.

BCR sequential extraction was used to compare the associations of Cd in the medium- and high-contaminated fields and this showed that 67-84% was extracted in BCR1, the exchangeable fraction. Tessier extraction was then used to distinguish

between Tessier-available and carbonate-bound forms of Cd and this revealed a significant difference between the medium and high-contaminated fields. Higher proportions (28-45%) of exchangeable Cd were found in medium-contaminated fields compared with only 22-24% in the high-contaminated fields. In contrast, 71-72% Cd was carbonate-bound in the high-contaminated fields but only 45-46% was in this form in the medium-contaminated fields. These results corroborate the proposed mechanisms of transfer of the carbonate-rich material from the mining area to the paddy fields.

6.4 Rice – Elemental Concentrations, Relationship with Soil and Extract Concentrations, Transfer Factors, and Limits for Human Consumption and Calculation of Typical Intake

Rice was collected from each of the paddy fields during the harvest period and was separated into roots, straw, husk and grain. In agreement with other field and laboratory studies, the highest concentrations were obtained for the roots while grain Cd as a percentage of root Cd concentrations ranged from only ~1.3-~3.6%. However, even for the low-contaminated fields, grain Cd concentrations of up to 0.6 mg kg^{-1} were obtained, exceeding the safe level for human consumption of 0.4 mg kg^{-1} (FAO/WHO, 2006). The highest rice grain Cd concentrations of 2.8-4.0 mg kg^{-1} were obtained for the fields with the highest total soil Cd contamination. Based on consumption of 0.28 kg rice per day and an average body weight of 60 kg for a 50 year old male, this corresponds to maximum values of ~20 µg Cd kg^{-1} body weight $week^{-1}$ and ~131 µg Cd kg^{-1} body weight $week^{-1}$ from consumption of rice grown in low- and high-contaminated fields respectively. Even for the former, this is ~3 times the safe level of 7 µg Cd kg^{-1} body weight $week^{-1}$ proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2010). Thus, consumption of rice grown in the study fields poses a significant long-term risk to human health.

It was therefore important to establish the underlying soil processes controlling uptake of Cd by the rice plants in order that a suitable remediative strategy could be developed. In the first instance, the relationships were established between grain Cd and (i) soil Cd; (ii) BCR-1 extractable Cd; and (iii) Tessier-1. There were strong correlations between the extractable Cd and grain Cd indicating the broad utility of either the BCR or Tessier method for estimating available Cd in these soils. As this finding was in contrast with previously published work by Simmons *et al.* (2005), it was decided to investigate the bioavailability of Cd via an independent method involving stable Cd isotopes.

6.5 Efficacy of Stable Isotope and Chemical Methods for Determining the Availability of Cd for Uptake by Rice Plants

A robust stable isotopic method was developed to determine the lability of Cd in the soils from medium- and high-contaminated fields. Comparing the E-value results from isotope exchange with those obtained using BCR-1 and Tessier-1 chemical extractions, it was clear that there was much closer agreement between the E values and the amount of Cd extracted in Tessier-1. Importantly, the differences between Cd availability in the medium- compared with the high-contaminated fields was again evident with lower E values (7-15 mg kg^{-1} vs 18-19 mg kg^{-1}) being obtained for the latter group. However, when the Tessier-1 extraction was carried out prior to determining the E values, there was a reduction of only 37-67% in available Cd. It was concluded that while neutral salt extraction (Tessier-1) may approximate the E value (or isotopically labile pool) for the soils under examination, the isotopically labile pool can have varying contributions from other fractions that are not directly accessed by the neutral salt extraction and the importance of such contributions may vary among different soils with varying properties (particularly in terms of pH, texture, carbonate content and cation exchange capacity). Thus future work should assess the relationship between the neutral salt and E value on a much wider variety

of soils. This would also allow determination of the degree to which the neutral salt extraction contributes to the isotopically exchangeable pool

6.6 Development of a Biochar Remediation Approach to Limit Cd Availability in Cd-contaminated Paddy Soils

Biochar was selected because of its recently reported potential to irreversibly bind potentially toxic metals such as Cd within soils. In relation to this study, biochar was produced from miscanthus and from rice husk because these are cheap, widely available substrates in Thailand. Based on the spectroscopic characterisation of the resulting chars, a production temperature of 350°C was preferred as this gave more oxygen-containing functional groups and higher cation exchange capacities. The chars themselves contributed very little exchangeable Cd (E values of 0.08-0.10 mg kg^{-1}) and were thus suitable for addition to the Cd-contaminated paddy soils. Comparing E values which have been corrected for total soil mass, a reduction of ~34% in available Cd was obtained by the addition of 20% w/w of either rice husk or miscanthus char (both produced at 350°C) to the medium-contaminated soils. However, a 5% addition of each char yielded reductions of ~16% and ~3% in available Cd and so the relationship between amount of char added and % reduction in availability was not linear and varied between RHC and MC.

Table 6.1 Calculated Amount of Char Required to Treat Cd-Contaminated Paddy Soils

Parameter	**Value**
Field group area	2,400 m^2
Depth of Contamination	0.4 m
Volume of Soil to Treat	960 m^3
Density of Loam Soil	1.36 g cm^{-3} = 1360 kg m^{-3}
Mass of Soil to Treat	1,305,600 kg
Mass of Char Required (20% addition)	261,120 kg = ~261 tonnes

Assuming a density of 1.36 g cm^{-3} for loam soils, ~261 tonnes of biochar would be required to treat the 0-40 cm soil for the entire field group (Table 6.1). However, the results for the high Cd-contaminated fields demonstrated that it would be inappropriate to include these in any such treatment strategy as no reduction in available Cd was achieved independent of the amount or type of char added to these soils. This was attributed to the low Tessier-exchangeable Cd and the predominant association of Cd with carbonate minerals in the high Cd-contaminated soils. Thus, only soils with a high proportion of available Cd, determined either by chemical extraction or using the E value method, were deemed to be suitable for biochar remediation. On the basis of the relationship observed between available Cd and grain Cd, a reduction in Cd availability by ~34% should reduce the intake due to consumption of rice to ~7 µg Cd kg^{-1} body weight $week^{-1}$ for low-contaminated fields and to ~19 µg Cd kg^{-1} body weight $week^{-1}$ for the medium-contaminated fields. This would bring the rice from 10 out of 18 fields to the safe level for consumption and a further 4 out of 18 to within three times this level. Thus it was concluded that it would be worthwhile proceeding with rice growth experiments involving soil with and without addition of biochar.

6.7 Development of a Procedure for Growing Rice on Cd-contaminated Paddy Soils under Laboratory-simulated Field Conditions

After many months of trials, the optimal method for growing rice in pots in an experimental facility involved germination of water-soaked rice seeds for 1-2 nights on wet paper toweling. The seedlings were then placed in germinating pots (containing clay soil) and were covered with whole grain rice husk char. After 1-2 months, the 12-15 cm plants were transferred to growing pots and submerged (2-3 cm) with water, except during fertilization (same rate as applied in the field). Throughout the growth period, the temperature in the growth tent was 22-27ºC, light intensity was 10,000-14,000 lux and the humidity was mostly 65-70%. After about four months, the soils were drained and allowed to dry out for 3-4 days.

Analysis of the RHC-amended medium- and high-Cd contaminated soils used for the rice growth experiments did not show the expected trends in E value that had been observed in the abiotic experiments. There was no effect of RHC addition on Cd availability for either the medium- or high-contaminated soils. Since the medium-contaminated soils had more exchangeable Cd and less carbonate-bound Cd, in agreement with the Tessier extractions results presented in Chapter 3 for soils from the same fields, it was concluded that the most likely reason was that the new batch of biochar used for the rice growth experiments may have had a lower sorption capacity for Cd. Thus further work should investigate the between-batch variability in the composition of biochar. This should include chars made from different batches of the source material, e.g. rice husk, and consecutive batches of char produced from the same batch of the source material.

Tessier sequential extraction of the RHC-amended medium-contaminated soils pre- and post-rice growth experiments revealed little change in soil Cd speciation in comparison with the control soils. Moreover, there was no discernible effect of rice

growth on Cd speciation within the control soil. A decrease in carbonate-bound Cd in the high-contaminated soils for the 5% RHC treatment had no effect on the available Cd as determined by the stable isotopic method.

From analysis of the pot-grown rice plants, it was evident that there were significant differences in the extent of Cd uptake compared with the field-grown rice plants. A greater extent of root uptake was observed for pot rice plants grown on the medium-contaminated soils whilst a lesser extent of uptake was observed for the high-contaminated soils. This emphasizes the difficulties in comparing results obtained for pot plant experiments with actual field conditions. Further work could focus on better simulating field conditions but it is likely that this would be a time-consuming and challenging task.

An encouraging finding was that there was a decrease in root uptake of Cd for the medium-contaminated soils with increasing addition of biochar. This merits further investigation in experiments that involve a wider range of biochar concentrations, e.g. up to 20% w/w, and a larger number of replicates in order that the merits of biochar remediation of Cd-contaminated paddy soils can be more robustly evaluated. An added dimension would be provided by rice growth experiments where rice grains suitable for harvesting have been produced.

6.8 Wider Implications of the Research Findings

From the research findings presented in this thesis, there are a number of key issues which are of importance for international investigations of the impacts of Cd on the environment and on human health. First of all, where Cd is released from a point source, e.g. mining activities, Cd transport and deposition in agricultural areas may continue even after waste management procedures have been improved or, indeed, mining activities have stopped. Although Cd concentrations in the truly dissolved

phase are likely to decrease, association with colloids and particulate matter may maintain or even increase the concentration of Cd being transported via receiving waters to downstream areas. Periods of increased rainfall, especially at the beginning of the monsoon season, increase soil erosion and enhance water loading with contaminated sediments. Mobilisation of contaminated aquatic sediments and transport further downstream is also enhanced during periods of high water flow. Researchers wishing to determine the full impact similar mining-related problems elsewhere must therefore take account of these continuing and seasonally influenced processes. Those wishing to alleviate the impact on agricultural land which is irrigated with mining-impacted waters should consider a sediment clean-up or dredging program (with appropriate waste disposal route) as this would reduce the likely transfer of Cd to the agricultural soils.

Secondly, although many studies, e.g. Simmons *et al.* (2005), have concluded that most of the Cd accumulated in the fields nearest to the source of contaminated irrigation water, i.e. the water creeks and canals, this study has shown that farming-related activities can also affect the Cd concentration in topsoil and change the expected pattern of contamination with increasing distance from the contaminated waterway. For example, in this study, the land owner carried out a one-off transfer of soil from higher-lying to lower-lying fields for irrigation purposes. Therefore, local knowledge of farming practices is considered to be essential in order to reach an informed interpretation of the processes controlling Cd distribution.

Thirdly, for soils with similar mineralogy to those in Thailand, the proxy for available Cd provided by Tessier-exchangeable or BCR1 (exchangeable) Cd should be used as a means of forecasting the Cd level in rice. The stable isotope exchange (E values) method also provides a valuable measure of Cd availability but the former techniques are cheaper and faster.

Lastly, for soil remediation approaches involving the addition of biochar, the total soil Cd and the ratio of available-to-total soil Cd were primarily good indicators of the success of the treatment. A “one-fits-all” approach to remediate Cd-contaminated soils should therefore not be encouraged. For example, from the results of this study, the total Cd >50 mg kg^{-1} with low percentage of available Cd (out of total Cd) could not amended by husk chars. However, in the medium-Cd soils (10-50 mg kg^{-1}), there was high percentage of available Cd in soils (~40%). With the rice husk chars amendment at 1 and 5% w/w, the rice root Cd was reduced by ~25 and ~30% and it was estimated that rice grain Cd would meet the safe level of 0.4 mg kg^{-1}. Biochar addition may therefore be a promising approach for both fertilizing and remediating Cd-contaminated soils where there is a high proportion of available Cd. There is a need, however, to develop a more suitable approach to remediate high Cd-contaminated paddy soils, particularly where there is a low proportion of available Cd, since the rice grown in these soils typically exceeds the safe levels for human consumption.

References

Padaeng Industry Public company limited. (2007) Annual report 2007. pp.17-18.

Simmons, R.W., P. Pongsakul,D. Saiyasitpanich, S. Klinhoklap, (2005) Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice downstream of a zinc mineralized area in Thailand: implication for public health. *Environmental Geochemistry and Health* 27(5-6): 501-511.

Appendix

Chapter 3

Appendix 3.1 Particle size analysis (Department of Sustainable Natural Resources, Australia)

Principle

Soil consists of an assembly of ultimate soil particles (discrete particles) of various shapes and sizes. The object of a particle size analysis is to group these particles into separate ranges of sizes and so determine the relative proportion by weight of each size range. The method employs sieving and sedimentation of a soil/water/dispersant suspension to separate the particles. The sedimentation technique is based on an application of Stokes' law to a soil/water suspension and periodic measurement of the density of the suspension.

Special apparatus

- Soil hydrometer (ASTM 152H hydrometer preferred).
- Uniform set of sedimentation cylinders with internal depth of 340±20 mm and capacity of 1 L. (See Note 1.)
- 0.20, 2.00 and 4.75 mm sieves.
- Interval timer.
- End-over-end shaker, rotating at 15 rpm.

Reagents

25% sodium hexametaphosphate (Calgon)

Dissolve 250 g of sodium hexametaphosphate in 900 mL of warm deionised water. When cool, add sufficient sodium carbonate to bring to pH 8 or 9 and dilute to 1 L with deionised water.

Sample preparation

Carry out sample preparation according to Sample Receipt, Preparation and Storage (S1A/5). Weigh and record the whole sample. Pass the sample through the 4.75 mm and 2 mm sieves making sure that no aggregates are retained on the sieve. Alternatively, the sample may be passed through a mechanical crusher with apertures of 2 mm to obtain the material <2 mm. Weigh and record the amount of particles >4.75 mm and 2.0 to 4.75 mm.

Sedimentation

1. On completion of shaking, transfer the prepared and dispersed samples to 1 L measuring cylinders. (See Note 1.) Fill to the 1 L mark with deionised water. Note the hydrometer used.
2. Stir with a plunger for 20–30 seconds ensuring that all material at the bottom is brought into suspension. At the end of stirring, remove the plunger and immediately start the interval timer.

3. After 4 minutes sedimentation, immerse the hydrometer to a depth slightly below its floating position and allow it to float freely. Take a reading at 5 minutes. Read at the top of the meniscus and record readings to the nearest 0.5 g/L.
4. Remove the hydrometer slowly, rinse clean and place in a sedimentation cylinder filled with deionised water and 20 mL of 25% sodium hexametaphosphate (blank solution). The water temperature in the blank cylinder must be the same as that of the soil suspension.
5. Re-insert the hydrometer in the soil suspension for readings at periods of 30, 93 and 420 minutes, taken in the same manner as above. If it is not possible to take a 420 minute reading, take a substitute reading at the end of the day, noting the elapsed time.
6. At about the same time as each soil suspension hydrometer reading, take a hydrometer and temperature reading (to the nearest 0.5°C) of the blank solution. Read the hydrometer at the top of the meniscus. The hydrometer should be left in the blank solution between readings.

Calculations
Calculate for each hydrometer reading the summation percentage (P).

$$P\,(\%) = \frac{(H - B)\,x\,100}{W}$$

Where:
H = Hydrometer reading in soil suspension (g/L)
B = Hydrometer reading in blank solution (g/L)

For the coarse sand fraction:

$$Coarse\ Sand\ (\%) = \frac{J\,x\,100}{\mathrm{W}}$$

Where:
J = Oven-dry (OD) weight of sand from sieve (g)

Where on a whole soil basis:

$$W = \frac{ODW\ x\ (G + ODWF)}{ODW}$$

Where:

ODW = OD weight of fine earth (<2.0 mm) used in hydrometer analysis (g)
J = OD weight of gravel (>2.0 mm) from sieve (g)
ODWF = OD weight of total fine earth (<2.0 mm) (g)

For the gravel fraction:

$$Gravel\ (\%) = G\ x\ 100\ /(G + ODWF)$$

Where:
G = Weight of gravel (>2.0 mm) from sieve (g)
ODWF = OD Weight of total fine earth (<2.0 mm) (g)

Calculate the particle size (D) at different times from the relationship:

$$D\ (mm) = 0.315\ x\ K\ x\ \sqrt{L\ /\ T}$$

Where:
K = Sedimentation constant, which varies with temperature and particle density
L = Tabulated effective length for the hydrometer reading (mm)
T = Time (minutes)

If the particle density of the soil is known, use that value in the K table. Otherwise, use a value of 2.65 Mg/m³. Values for K for a range of temperatures and density are listed in handbook (see the reference in chapter 3).

Plot cumulative percentage (P) versus particle size diameter (D) on a semi-logarithmic, 4 cycle graph paper. Interpolate from the curve the percentage for:

clay = <0.002 mm
silt = 0.002–0.02 mm
fine sand = 0.02–0.20 mm
coarse sand = 0.20–2.0 mm
gravel = >2.0 mm

Appendix 3.2 Cd Concentrations in Size Fractions from Creek (2 samples) and Canal (4 samples) Samples Collected During the Dry Period (July 2010)

Sediment size fraction	% mass	Cd concentration (mg kg^{-1})	% of total sediment Cd
53 µm - 2 mm			
Cr1	32.0	19.0±0.87	27.6
Cr2	28.6	19.5±1.42	25.6
Ca1	21.2	30.8±1.13	17.5
Ca2	15.0	26.5±1.00	21.3
Ca3	22.4	36.3±0.99	31.5
Ca4	39.2	12.6±0.91	19.6
20 - 53 µm			
Cr1	47.9	7.2±0.68	24.8
Cr2	50.7	9.6±0.85	30.5
Ca1	63.7	23.2±3.00	55.2
Ca2	72.0	12.3±0.07	54.9
Ca3	60.3	10.4±1.04	31.5
Ca4	49.7	4.1±0.69	28.1
<20 µm			
Cr1	20.1	20.55±1.32	47.6
Cr2	20.8	24.37±1.45	43.9
Ca1	15.1	34.12±1.15	27.3
Ca2	13.0	25.76±0.28	23.8
Ca3	17.3	32.87±1.20	37.0
Ca4	11.1	9.48±0.20	52.3

Appendix 3.3 Cd Concentration of Sediments Calculated from the sum of Cd Concentrations and Mass of 3 Size Fractions (samples collected during dry period)

Site	Distance (m) from Cr1	Cd concentration (3 size fractions) (mg kg^{-1})	Weight (g)	Total Cd concentration (mg kg^{-1})
Cr1	-	2.98	0.04	13.8
Cr2	112	3.51	0.06	15.8
Main check dam	115			
Ca1	276	8.22	0.22	26.7
Ca2	724	8.13	0.13	16.2
Ca3	760	8.28	0.16	19.9
Small check dam	767			
Ca4	1,089	7.88	0.06	7.14

Appendix 3.4 Mineral Concentrations from Each Topsoil (0-20 cm) of the 18 Fields (mean of duplicate ± standard error; in mg kg^{-1})

Fields	Cu	Fe ($g\ kg^{-1}$)	Mn	Pb	Zn	Cd	Ca ($g\ kg^{-1}$)	Mg	P	S
F1	17.6±0.7	21.3±0.4	896±68.5	36.0±0.7	274±3.3	4.67±0.1	15.4±0.2	357±32.2	247±6.3	247±20.0
F2	17.3±0.1	19.2±0.6	765±22.5	30.0±0.1	150±0.9	2.48±0.1	14.9±0.1	1,016±47.9	260±4.5	118±5.1
F3	15.6±0.1	19.8±0.2	563±0.9	33.1±0.5	268±0.5	4.46±0.02	14.8±0.02	367±22.3	242±1.8	195±3.2
F4	18.3±0.2	16.5±0.3	317±0.8	55.2±0.1	590±2.9	16.8±0.5	14.8±0.2	117±47.8	270±2.8	187±3.6
F5	18.4±0.2	16.7±0.01	759±0.4	34.5±0.4	182±0.9	4.10±0.01	14.1±0.03	71.4±6.17	242±1.4	126±0.4
F6	13.1±0.2	14.8±0.1	438±0.2	29.7±0.2	173±0.2	3.89±0.02	14.0±0.01	47.0±12.4	201±1.7	115±3.9
F7	20.4±0.4	17.3±0.4	522±0.2	34.8±0.2	174±2.7	3.70±0.1	14.2±0.03	102±8.5	265±9.3	136±1.1
F8	15.4±0.1	16.8±0.2	601±0.4	32.7±0.4	164±1.7	3.57±0.03	13.9±0.01	62.8±6.13	215±2.2	120±2.8
F9	17.0±0.6	15.5±0.4	340±6.8	68.3±1.3	1,023±27.8	30.8±1.8	17.8±0.1	624±5.3	273±6.7	269±0.02
F10	15.9±0.3	14.0±0.2	758±4.4	129±4.4	2,590±31.6	83.7±0.4	27.4±0.04	3,055±200	237±4.3	521±8.1
F11	20.2±0.3	17.3±0.3	362±0.1	39.1±0.1	336±1.4	8.74±0.05	15.0±0.01	1,433±110	320±1.5	170±1.2
F12	13.9±0.002	13.3±0.4	298±0.6	26.0±0.6	125±2.1	2.57±0.1	14.0±0.04	240±35.7	201±3.3	113±2.0
F13	13.8±1.1	14.3±1.2	288±2.6	27.3±2.6	141±12.0	2.87±0.3	14.0±0.02	274±31.3	191±15.5	122±6.4
F14	22.6±1.0	18.58±1.3	730±170	73.3±3.2	1,042±87.0	35.4±4.3	18.1±0.3	1,654±152	329±16.4	276±11.8
F15	49.2±21.0	20.9±1.1	879±6.8	125±6.8	2,093±140	74.6±5.4	24.6±0.6	3,944±180	401±14.8	512±19.2
F16	16.4±0.5	14.1±0.3	607±3.0	84.1±3.0	1,432±33.8	47.0±0.7	21.8±0.03	2,024±238	236±8.4	365±8.1
F17	21.5±0.6	17.1±0.8	971±8.4	142±8.4	2,534±161	87.6±8.6	28.0±1.1	4,013±324	295±23.1	555±43.0
F18	17.3±1.3	14.3±0.9	705±9.3	126±9.3	2,330±196	81.9±4.7	29.0±1.0	3,613±242	242±19.4	568±38.4
Thai*	14.9	29.3	340	13.7	29	0.03	3.0	2,800	38	65

* Background values for Cd in Thai soils, Prakongkep *et al*., 2008

Appendix 3.5 Cd Concentrations in Rice Grain from Each of the 18 Fields (mean ± standard error; n=2; in mg kg^{-1})

Fields	Cd concentration (mg kg^{-1})
F1	0.07±0.02
F2	0.05±0.00002
F3	-*
F4	0.90±0.00002
F5	0.40±0.001
F6	0.56±0.02
F7	0.12±0.02
F8	0.22±0.02
F9	0.54±0.05
F10	4.03±0.04
F11	0.49±0.003
F12	0.19±0.001
F13	0.29±0.002
F14	0.37±0.02
F15	0.65±0.003
F16	1.19±0.005
F17	2.89±0.04
F18	2.77±0.02

* Rice was harvested before sample collection.

Chapter 5

Appendix 5.1 Trial and Error Process of Germination and Rice Growing Trials

In late December 2012, the rice growing started in the laboratory. The tent and accessories were set up. Numerous germinating trials were carried out. Some involved slight adjustments, while some involved major changes. The major changes are reported in this Appendix. The first attempt at rice germination started by stimulating rice seeds sown on clay-rich soil in the germinating plastic box which was covered with clear plastic sheet in order to keep the moisture high. However the rice did not grow well. This method was repeated many times but the results were the same. The second attempt changed the germinating soils to a mixture of sand, whole grain rice husk chars (WG-RHC) and clay-rich soil. The rice grew quite well and the seedlings were transplanted with bare roots to the pots when they were 2-3 weeks old. However, the transplanted rice did not grow well and again repeated attempts gave similar results.

During the third attempt, the rice seeds were germinated only on WG-RHC. The rice seedlings did not develop very well. When bare root seedlings were moved to the experimental pots, the young rice plants stopped developing and died. The fourth attempt involved germination in a thin layer of WG-RHC above the clay paddy soil. This produced good results. The roots penetrated to the clay layer and the young rice plants developed quite well. When the seedlings were about two weeks old, they were transplanted to the pot with bare roots. The rice plants grew well in the first few weeks but then stopped growing and died after 1-2 months. This method was repeated many times whilst the conditions in the tent were adjusted but the results were similar. Next, a seed germination tray with many tiny pots was trialled and used as a substitute for the plastic germinating box. The use of clay-rich soil with whole grain RHC on top again resulted in healthy young rice plants but transplantation with bare roots, i.e. no soils attached again resulted in failure. This led to some trial and error regarding the way that the young rice plants were transplanted with soils from

the small pocket (in the seed germination tray) where they had been growing well. It was clear that transferring the plants with soil attached to the roots was more successful. In summary, two techniques were found to be useful for the full rice-growing trials. These were (i) germinating on clay with a thin layer of WG-RHC and (ii) transplanting seedlings with soil attached to the roots.

Towards summer 2013, the ambient temperature in Edinburgh was higher and so it was easier to maintain the required rice-growing temperature of 22-27°C. An attempt was made to grow the rice directly in the experimental pots thus avoiding the need for transplantation. Here polystyrene cups with bottoms removed were placed at a depth of 2 cm in the centre of each pot. Inside, the cup was filled up with the rice seeds in clay soil which was covered with a thin layer of the chars. Then the rice seeds were germinated in the polystyrene cups. The rice developed well but again stopped growing when 1-2 months old. In some pots, it was found that the roots could not penetrate through the WG-RHC to the clay layer and that caused stunted plant growth.

The last attempt was germinating the rice seeds in the pots (the same size as the rice growing pot) which were filled with the WG-RHC layer and clay. Then the young rice was allowed to grow until it appeared healthy at about 1-2 months age. After that seedlings were transplanted with soils attached into the rice growing pots. This method was more successful than all the previous ones as the rice grew healthily for longer than 2 months. Unfortunately, the project time ended at this point and so it was not possible to see whether or not rice grains were produced.